PATENT
55-301M

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re : U.S. Patent 4,604,463

Issued : August 5, 1986

To : Tadashi MIYASAKA, Seigo SAWADA, Kenichiro NOKATA, Eiichi SUGINO, and Masahiko MUTAI

Assignee : KABUSHIKI KAISHA YAKULT HONSHA

For : CAMPTOTHECIN DERIVATIVES AND PROCESS FOR PREPARING SAME

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

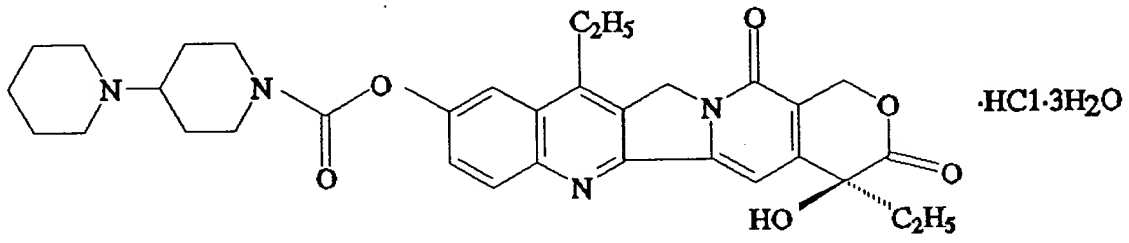
Commissioner of Patents
and Trademarks
Washington, D. C. 20231

Dear Sir:

Your applicant, KABUSHIKI KAISHA YAKULT HONSHA, represents that it is the assignee of the entire interest in and to Letters Patent for the United States 4,604,463 granted to Tadashi Miyasaka, Seigo Sawada, Kenichiro Nokata, Eiichi Sugino and Masahiko Mutai, on August 5, 1986 for CAMPTOTHECIN DERIVATIVES AND PROCESS FOR PREPARING SAME by virtue of an Assignment in favor of KABUSHIKI KAISHA YAKULT HONSHA recorded on July 5, 1984, at Reel 4301, Frames 948-949. Your applicant, acting through its duly authorized attorney whose power to act on behalf of applicant is filed simultaneously herewith (Exhibit 1), hereby submits this application for extension of patent term under 35 U.S.C. 156 by providing the following information required by 37 C.F.R. 1.740. For convenience, the information contained in this application will

be presented in a format and order which follows the requirements of 37 C.F.R. 1.740.

(1) The approved product CAMPTOSAR® (also referred to in some correspondence as "CPT-11") contains Irinotecan hydrochloride trihydrate, chemically described as (1) 7-Ethyl-10-[(4-piperidino-piperidino)carbonyloxy]-camptothecin hydrochloride trihydrate or (2) 7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin hydrochloride trihydrate. Its structural formula is:



(2) The approved product was subject to regulatory review under the Federal Food, Drug and Cosmetic Act, § 512.

(3) The approved product CAMPTOSAR® received permission for commercial marketing or use under § 512 of the Federal Food, Drug and Cosmetic Act by virtue of a letter sent by the FDA dated June 14, 1996.

(4) The only active ingredient in CAMPTOSAR® is Irinotecan hydrochloride trihydrate, which has not been approved for

commercial marketing or use under § 512 of the Federal Food, Drug and Cosmetic Act prior to approval of NDA 20-571 by the Food and Drug Administration on June 14, 1996.

(5) This application for extension of patent term under 35 U.S.C. § 156 is being submitted within the permitted 60 day period pursuant to 37 C.F.R. 1.720(f), which period will expire on August 13, 1996.

(6) The complete identification of the patent for which a term extension is being sought is as follows:

Inventors: Tadashi Miyasaka, Seigo Sawada, Kenichiro Nokata, Eiichi Sugino and Masahiko Mutai

Patent No.: 4,604,463

Issue Date: August 5, 1986

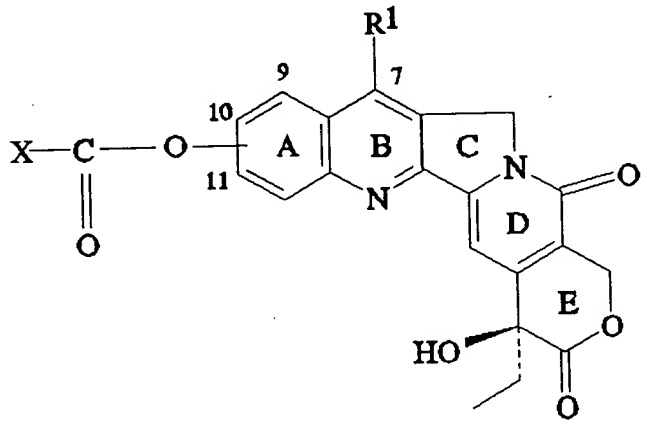
Expiration Date: July 5, 2004 (20 years from filing date)

(7) A true copy of the patent is attached as Exhibit 2.

(8) No Terminal Disclaimer, Certificate of Correction or Reexamination Certificate has been issued. Enclosed are copies of the receipts verifying payment of the maintenance fees in 1990 and 1994 (see Exhibit 3).

(9) U.S. Patent 4,604,463 claims the active compound of CAMPTOSAR®. Claims 1 and 20 of the patent claim camptothecin derivatives as follows:

Claim 1. Camptothecin derivatives of the formula:



(I)

wherein R^1 is a hydrogen atom, a halogen atom or an alkyl group with 1-4 carbon atoms and X is a chlorine atom or $-NR^2R^3$ where R^2 and R^3 are the same or different and each represents a hydrogen atom, a substituted or unsubstituted alkyl group with 1-4 carbon atoms or a substituted or unsubstituted group selected from the group consisting of cyclopentyl, cyclohexyl, N-methylpiperidyl-(4), 2-pyrrolidyl, phenyl, tolyl, xylyl, pyridyl-2 and 2-methylpyridyl-(4), with the proviso that when both R^2 and R^3 are the substituted or unsubstituted alkyl

groups, they may be combined together with the nitrogen atom, to which they are bonded, to form a heterocyclic ring selected from the group consisting of pyrrolidine, piperidine, 2-oxapyrrolidine, morpholine, thiomorpholine and 4-R^4 piperizine rings in which R^4 is a hydrogen atom, a substituted or unsubstituted alkyl group with 1-4 carbon atoms or a substituted or unsubstituted phenyl group and wherein the grouping -O-CO-X is bonded to a carbon atom located in any of the 9-, 10- and 11-positions in the ring A, and ammonium salts or alkali metal salts thereof.

Claim 20. Camptothecin derivatives according to claim 1, which are 10-[4-(piperidino)-1-piperidino]carbonloxy-7-R^1-camptothecins.

The claims read on the active compound of the approved product CAMPTOSAR®. The active ingredient of CAMPTOSAR®, Irinotecan hydrochloride trihydrate, is an antitumor compound. Irinotecan hydrochloride trihydrate is chemically described as (1) 7-Ethyl-10-[(4-piperidino-piperidino)carbonyloxy]-camptothecin hydrochloride trihydrate or (2) 7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin hydrochloride trihydrate. This hydrochloride is water soluble and yields (1) 7-Ethyl-10-[(4-piperidino-piperidino)carbonyloxy]-camptothecin or (2) 7-Ethyl-10-

[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, the active compound for exhibiting antitumor activity.

(10) Relevant dates and information pursuant to 35 U.S.C. § 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review are as follows:

An Investigational New Drug application (IND 35,229) for CPT-11 (Irinotecan hydrochloride trihydrate) was filed on August 3, 1990 and became effective on September 5, 1990.

A New Drug Application (NDA 20-571) for CAMPTOSAR® was submitted on December 28, 1995.

The New Drug Application (NDA 20-571) for CAMPTOSAR® was approved on June 14, 1996.

(11) As a brief description of the activities undertaken by applicant or applicant's representatives during the applicable regulatory review period, attached hereto is a chronology of the major communications between the applicant and the FDA from August 3, 1990 to June 14, 1996. (See Exhibit 4).

It will be noted that the initial IND Application for CPT-11 was filed on August 3, 1990 by G.H. Besselar Associates as authorized agent for KABUSHIKI KAISHA YAKULT HONSHA. The authorized representative for the IND was subsequently transferred to Theradex. Sponsorship of the IND was then later transferred to the Upjohn Company who subsequently filed the NDA for CAMPTOSAR®/CPT-11 on December 28, 1995. Finally, on June 11, 1996, the Upjohn Company changed its name to "The Pharmacia and Upjohn Company", which is now the owner of the NDA 20-571.

(12)(i) Applicant is of the opinion that U.S. Patent 4,604,463 is eligible for extension under 35 U.S.C. § 156 because it satisfies all requirements for extension as follows:

(a) 35 U.S.C. § 156(a) - U.S. Patent 4,604,463 claims as a new compound the active ingredient in CAMPTOSAR®.

(b) 35 U.S.C. § 156(a)(1) - U.S. Patent 4,604,463 has not expired before submission of this application.

(c) 35 U.S.C. § 156(a)(2) - The term of U.S. Patent 4,604,463 has never been extended under 35 U.S.C. § 156(e)(1).

(d) 35 U.S.C. § 156(a)(3) - The application for extension is submitted by the owner of record of the patent in accordance with the requirements of paragraphs (1) through (4) of 35 U.S.C. § 156(d) and the rules of the Patent and Trademark Office.

(e) 35 U.S.C. § 156(a)(4) - The product CAMPTOSAR® has been subjected to a regulatory review period before its commercial marketing or use.

(f) 35 U.S.C. § 156(a)(5)(A) - The commercial marketing or use of the product CAMPTOSAR® after the regulatory review period is the first permitted commercial marketing or use under the provision

of the Federal Food, Drug and Cosmetic Act (i.e. Section 512) under which such regulatory review period occurred.

(g) 35 U.S.C. § 156(c)(4) - No other patent has been extended for the same regulatory review period for the product CAMPTOSAR®.

(12)(ii) The length of the extension of patent term of U.S. Patent 4,604,463 claimed by Applicant is 1,139 days or 3.12 years. The length of the extension was determined pursuant to 37 C.F.R. § 1.778 as follows:

(a) The regulatory review period under 35 U.S.C. § 156(g)(4)(B) began on September 5, 1990 and ended June 14, 1996, which is a total of 2,109 days or 5.78 years, which is the sum of (1) and (2) below:

(1) The period of review under 35 U.S.C. § 156(g)(4)(B)(i), the "Testing Period", began on September 5, 1990 and ended on December 28, 1995, which is 5.32 years or 1,940 days.

(2) The period of review under 35 U.S.C. § 156(g)(4)(B)(ii), the "Approval Period", began on December 28, 1995 and ended on June 14, 1996, which is 0.46 years or 169 days.

(b) The regulatory review period upon which the period of extension is calculated is the entire regulatory review period as determined in subparagraph (12)(ii)(a) above (2,109 days) less:

(1) The number of days in the regulatory review period which were on or before the date on which the patent issued (August 5, 1986) which is zero (0) days; and

(2) The number of days during which applicant did not act with due diligence, which is zero (0) days; and

(3) One-half the number of days determined in subparagraph (12)(ii)(a)(1) after the patent issued (one-half of 1,940 days) which is 970 days;

(c) The number of days as determined in subparagraph (12)(ii)(b) (1,139 days or 3.12 years) when added to the original term of the patent (July 5, 2004) would result in the date August 15, 2007;

(d) Fourteen (14) years when added to the date of NADA approval June 14, 1996 would result in the date June 14, 2010;

(e) The earlier date as determined in subparagraphs (12)(ii)(c) and (12)(ii)(d) is August 15, 2007.

(f) Since U.S. Patent 4,604,463 issued after September 24, 1984, the period of extension may not exceed five (5) years. Five (5) years when added to the original expiration date of the patent (July 5, 2004) would result in the date of July 5, 2009.

(g) The earlier date as determined by subparagraph (12)(ii)(e) and (12)(ii)(f) is August 15, 2007.

(13) Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

(14) The prescribed fee under 37 C.F.R. § 1.20(j) for receiving and acting upon this application is attached as a check in the amount of $1,060.00. The Commissioner is authorized to charge any additional fees required by this application to Deposit Account No. 02-2448.

(15) All correspondence and inquiries may be directed to the undersigned, whose address, telephone number and fax number are as follows:

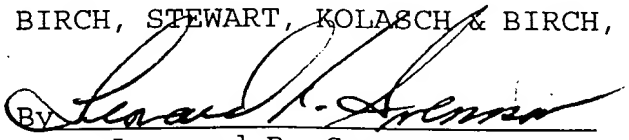
Leonard R. Svensson
BIRCH, STEWART, KOLASCH & BIRCH, LLP
P. O. Box 747
Falls Church, VA 22040-0747
Phone: (703) 205-8000
Fax: (703) 205-8050

(16) Enclosed is a certification that the application for extension of patent term under 35 U.S.C. § 156 including its attachments and supporting papers is being submitted as one original and a duplicate copy thereof (Exhibit 5).

(17) The requisite Declaration pursuant to 37 C.F.R. § 1.740(b) is attached (Exhibit 6).

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 
Leonard R. Svensson
Reg. No. 30,330

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

Dated: August 12, 1996

Attachments:
Power of Attorney (Exhibit 1)
U.S. Patent 4,604,463 (Exhibit 2)
Copies of Receipts for Maintenance Fees (Exhibit 3)
Chronology of Regulatory Review Period (Exhibit 4)
Certification of Copies of Application Papers (Exhibit 5)
Declaration pursuant to 37 C.F.R. § 1.740(b) (Exhibit 6)

LRS/pw

EXHIBIT 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee : Tadashi MIYASAKA et al.

Patent No.: 4,604,463

Issued : August 5, 1986

To : Tadashi MIYASAKA, Seigo SAWADA, Kenichiro NOKATA, Eiichi SUGINO, and Masahiko MUTAI

Assignee : KABUSHIKI KAISHA YAKULT HONSHA

For : CAMPTOTHECIN DERIVATIVES AND PROCESS FOR PREPARING SAME

POWER OF ATTORNEY AND APPOINTMENT OF AGENT PURSUANT TO 37 C.F.R. § 1.730

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

Assignee, KABUSHIKI KAISHA YAKULT HONSHA, a corporation organized and existing under the laws of Japan, represents that it is the Assignee of the entire right, title, and interest in and to United States Letters Patent 4,604,463 by virtue of an Assignment to KABUSHIKI KAISHA YAKULT HONSHA recorded on July 5, 1984 at Reel 4301, Frames 948-949.

Assignee, KABUSHIKI KAISHA YAKULT HONSHA, as owner of record of the above-identified United States Letters Patent, hereby appoints BIRCH, STEWART, KOLASCH & BIRCH, LLP, and Raymond C. Stewart (Reg. No. 21,066), Terrell C. Birch (Reg. No. 19,382), Joseph A. Kolasch (Reg. No. 22,463), Anthony L. Birch (Reg. No. 26,122), James M. Slattery (Reg. No. 28,380), Bernard L. Sweeney

(Reg. No. 24,448), Michael K. Mutter (Reg. No. 29,680), Charles Gorenstein (Reg. No. 29,271), Leonard R. Svensson (Reg. No. 30,330), Gerald M. Murphy, Jr. (Reg. No. 28,977), Terry L. Clark (Reg. No. 32,644), Marc S. Weiner (Reg. No. 32,181), Andrew D. Meikle (Reg. No. 32,868), Andrew F. Reish (Reg. No. 33,443), Joe McKinney Muncy (Reg. No. 32,334), and C. Joseph Faraci (Reg. No. 32,350), as its attorneys pursuant to 37 C.F.R. § 1.730 to conduct all business before the United States Patent and Trademark Office relative to an Application for Patent Term Extension pursuant to 35 U.S.C. § 156 for the above-identified United States Letters Patent.

Please send all future correspondence concerning the above matter to BIRCH, STEWART, KOLASCH & BIRCH, LLP, at the following address:

Post Office Box 747
Falls Church, Virginia 22040-0747
Telephone: (703) 205-8000
Facsimile: (703) 205-8050

KABUSHIKI KAISHA YAKULT HONSHA

DATED: August 6, 1996 S. Hori

Sumiya HORI
Name

President
Title

United States Patent [19]

Miyasaka et al.

[11] **Patent Number: 4,604,463**

[45] **Date of Patent: Aug. 5, 1986**

8/5/2003

[54] CAMPTOTHECIN DERIVATIVES AND PROCESS FOR PREPARING SAME

[75] Inventors: **Tadashi Miyasaka**, Kanagawa; **Seigo Sawada**, Tokyo; **Kenichiro Nokata**, Tokyo; **Eiichi Sugino**, Tokyo; **Masahiko Mutai**, Tokyo, all of Japan

[73] Assignee: **Kabushiki Kaisha Yakult Honsha**, Tokyo, Japan

[21] Appl. No.: **627,980**

[22] Filed: **Jul. 5, 1984**

[30] **Foreign Application Priority Data**

Jul. 14, 1983 [JP] Japan 58-126946

[51] **Int. Cl.**[4] **C07D 491/22**

[52] **U.S. Cl.** **544/125**; 544/60; 544/361; 546/48

[58] **Field of Search** 546/48; 544/361, 125, 544/60

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,894,029	7/1975	Winterfeldt et al.	546/48
4,031,098	6/1977	Sugasawa	546/48
4,399,276	8/1983	Miyasaka et al.	546/48
4,399,282	8/1983	Miyasaka et al.	546/48
4,473,692	9/1984	Miyasaka et al.	546/48

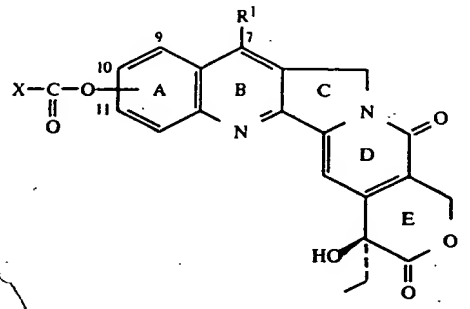
FOREIGN PATENT DOCUMENTS

74770	3/1983	European Pat. Off.	546/48
74256	3/1983	European Pat. Off.	546/48
88642	8/1983	European Pat. Off.	546/48
154583	9/1983	Japan	546/48
154584	9/1983	Japan	546/48
51289	3/1984	Japan	546/48

Primary Examiner—George F. Lesmes
Assistant Examiner—S. A. Gibson
Attorney, Agent, or Firm—Birch, Stewart, Kolasch & Birch

[57] **ABSTRACT**

New camptothecin derivatives possessing high antitumor activity with slight toxicity, represented by the general formula:



(I)

wherein R^1 is a hydrogen atom, a halogen atom or an alkyl group with 1–4 carbon atoms and X is a chlorine atom or $—NR^2R^3$ where R^2 and R^3 are the same or different and each represents a hydrogen atom, a substituted or unsubstituted alkyl group with 1–4 carbon atoms or a substituted or unsubstituted carbocyclic or heterocyclic group, with the proviso that when both R^2 and R^3 are the substituted or unsubstituted alkyl groups, they may be combined together with the nitrogen atom, to which they are bonded, to form a heterocyclic ring which may be interrupted with —O—, —S— and/or $>N—R^4$ in which R^4 is a hydrogen atom, a substituted or unsubstituted alkyl group with 1–4 carbon atoms or a substituted or unsubstituted phenyl group and wherein the grouping —O—CO—X is bonded to a carbon atom located in any of the 9-, 10- and 11-positions in the ring A of camptothecin, as well as an ammonium salt or an alkali metal salt thereof. These new camptothecin derivatives are prepared by reacting a 7-R^1-camptothecin derivative having a hydroxyl group in any of the 9-, 10- and 11-positions on the ring A thereof with phosgen and then reacting, if necessary, the resultant 7-R^1-camptothecin derivative having a chlorocarbonyloxy group in the same position on the ring A thereof with an amine HNR^2R^3 or by reacting a 7-R^1-camptothecin derivative having a hydroxyl group in any of the 9-, 10- and 11-positions on the ring A thereof with a carbamoyl chloride Cl-$CONR^2R^3$.

25 Claims, No Drawings

CAMPTOTHECIN DERIVATIVES AND PROCESS FOR PREPARING SAME

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to new camptothecin derivatives possessing anti-tumor activity and to a process for preparing such derivatives. More particularly, the present invention relates to new camptothecin derivatives carrying an aminocarbonyloxy group or a chlorocarbonyloxy group in any of the 9-, 10- and 11-positions on the ring A thereof and possessing excellent anti-tumor activity with a low level of toxicity as well as a process for the preparation of the new camptothecin derivatives.

2. Description of the Prior Art

Camptothecin is an alkaloid extracted and isolated from *Camptotheca accuminata* (Nyssaceae), etc., which has a pentacyclic structure consisting of a characteristic fused 5-ring system consisting of quinoline (rings A and B), pyrroline (ring C), α-pyridone (ring D) and a six-membered lactone (ring E) and is distinguished by displaying a strong inhibitory activity toward biosynthesis of nucleic acid. In addition, camptothecin is a unique anti-tumor substance characterized by its rapid and reversible action, its lack of any cross-tolerance with the existing anti-tumor agents and by exhibiting a strong anti-tumor activity against experimentally transplanted carcinoma such as leukemia L-1210 in mice or Walker 256 tumor in rats. Although camptothecin is still regarded as one of the most potent substances possessing anti-tumor activity, the use of this compound itselt for clinical treatments is significantly limited because of high toxicity. Moreover, camptothecin and the majority of derivatives thereof are sparingly soluble in water and thus involve a problem in case of administration as medicaments.

Accordingly, a number of attempts have been made not only to reduce toxicity of camptothecin while maintaining its anti-tumor activity by converting camptothecin chemically into its derivatives but also to make camptothecin and derivatives thereof easily soluble in water by chemical modifications of the camptothecin molecule or substituents therein. The chemical modifications so far reported are mainly about the ring D and/or E of camptothecin. As a method for making camptothecin or derivatives thereof soluble in water, for example, a ring-opening reaction for the E-ring (lactone ring) of camptothecin was used in the prior arts to form an alkali metal salt of the carboxyl function. However, any chemical modification of the ring D and/or E, including such ring-opening reaction, revealed only failure in maintaining anti-tumor activity and very poor improvement in toxicity [J. Med. Chem., 19 (1976), 675]. From the chemotherapeutic point of view, therefore, it is of importance that the chemical modifications of camptothecin should be restricted in the rings A, B and C without effecting any change in the rings D and E which are believed to be the essential structural elements for the expression of the above mentioned characteristic biological activities.

Except for a method for functionalizing the 12-position of camptothecin reported in 1976 which comprises a series of many troublesome conversion and purification operations starting with a relatively easily operable nitration at the 12-position [P. Pei-chuang et al., Hau Hsueh Hsueh Pao 33 (1975), 71; Chem. Abstr. 84 (1976) 115629p], no success was reported until 1979 in connection with chemical functionalization of camptothecin in a moiety involving the rings A, B and C. This is probably ascribable to the reasons that camptothecin itself is only sparingly soluble in various organic solvents and that camptothecin possessing the molecular nature of heterocyclic rings is resistant to the so-called electrophilic reactions conventionally carried out on aromatic rings. In the present status, such obstacles strongly discourage chemical modifications of camptothecin contemplated academically for preparing new classes of derivatives thereof.

Under the above mentioned circumstances, the present inventors previously found together with co-workers processes for preparing 5- and 7-substituted camptothecins (U.S. Pat. No. 4,399,282) by introducing (1) a hydroxymethyl group into the 7-position of camptothecin by subjecting camptothecin to a radical reaction with methanol by the aid of sulfuric acid and a peroxide, (2) a hydroxy group into the 5-position of camptothecin by treating camptothecin with sulfuric acid, water and a persulfate in the presence of a metal ion or by treating camptothecin with iodine in the presence of a base, and (3) an alkyl or aralkyl group into the 7-position of camptothecin by subjecting camptothecin to a radical reaction with a compound of the general formula: RX

(wherein R stands for an alkyl group or an aralkyl group, and

X for $-CH_2OH$, $-COOH$, $-CHO$, $-CO-R$ or $-\overset{R}{\underset{R}{C}}-OOH$)

preferably in a large excess amount by the aid of sulfuric acid, water and a peroxide in the presence of a metal ion. Further, the present inventors prepared together with co-workers a great number of new camptothecin derivatives from these 5- and 7-substituted camptothecin derivatives (U.S. Pat. Nos. 4,399,276 and 4,399,282) according to the process wherein 7-hydroxymethylcamptothecin is acylated with an acylating agent to obtain 7-acyloxymethylcamptothecins or 20-O-acyl-7-acyloxymethylcamptothecins or wherein 7-hydroxymethylcamptothecin is oxidized with an oxidizing agent usually capable of oxidizing a hydroxymethyl group to a carboxyl group to obtain 7-carboxycamptothecin, which is then esterified with an alcohol to obtain 7-alkoxycarbonylcamptothecins, the process wherein 5-alkoxycamptothecins are obtained by dissolving 5-hydroxycamptothecin in a lower alcohol, adding thereto an acid and heating the mixture, or wherein 5-acyloxycamptothecins or 20-O-acyl-5-acyloxycamptothecins are obtained by acylating 5-hydroxycamptothecin with a reactive acid derivative such as an acid anhydride or a halide of a carboxylic acid, the process wherein camptothecin-7-aldehyde is obtained by treating the 7-hydroxymethylcamptothecin with various cationoid reagents without using any oxidizing agent, and the process wherein 7-alkoxymethylcamptothecins and 7-dialkoxymethylcamptothecins are obtained by treating 7-hydroxymethylcamptothecin in a lower alkanol or an aralkyl alcohol with an acid.

Further successively, the present inventors prepared together with co-workers camptothecin-1-oxide or 7- or 5-substituted derivatives thereof by treating camptothecin or a 7- or 5-substituted derivative thereof with an

N-oxidizing agent (Japanese Laid-open Patent Appln. No. 58-39685; U.S. Ser. No. 414,528) as well as various 10-substituted camptothecin derivatives according to the process wherein camptothecin-1-oxide or a 7- or 5-substituted derivative thereof obtained as above is reacted with an active hydrogen-containing reagent in the presence of an acid under irradiation of UV-rays (Japanese Laid-open Patent Appln. No. 58-39683; U.S. Ser. No. 413,879) or the process wherein camptothecin is first catalytically hydrogenated and the resultant 1,2,6,7-tetrahydrocamptothecin is treated with an acylating agent and then with a mixture of nitric acid and sulfuric acid to form a 1-acyl-10-nitro-1,2,6,7-tetrahydrocamptothecin which is then deacylated and oxidized to 10-nitrocamptothecin and is then modified in various manners known per se to convert the 10-nitro group into other 10-substituents, e.g. 10-hydroxy group (Japanese Laid-open Patent Applns. Nos. 58-134095 and 58-152888; U.S. Ser. No. 413,879).

It was also found that when camptothecin in sulfuric acid was treated carefully with nitric acid under ice-cooling and agitation, new 9-nitrocamptothecin could be obtained in a yield of 30–40% together with the known 12-nitrocamptothecin. This new 9-nitrocamptothecin is then reduced to 9-aminocamptothecin which can be converted into various 9-substituted camptothecin derivatives according to the methods known per se as in the case of 10-nitrocamptothecin (Japanese Laid-open Patent Appln. No. 59-51289). For example, 9-aminocamptothecin can be converted into the corresponding 9-halogeno or 9-cyano derivative by once converting the 9-amino derivative into 9-diazonium salt and then treating the salt with cuprous halide or cyanide according to the Sandmayer reaction. Further, 9-diazonium salt can be converted into 9-hydroxy, 9-alkoxy and 9-acyloxy derivatives in the manner known per se.

Various 11-substituted camptothecin derivatives were prepared by catalytically hydrogenating camptothecin to form 1,2,6,7-tetrahydrocamptothecin, treating this tetrahydro derivative directly with sulfuric acid and nitric acid whereby the 11-position of camptothecin was selectively nitrified to form 11-nitro-1,2,6,7-tetrahydrocamptothecin, oxidizing the 11-nitro- 1,2,6,7-tetrahydrocamptothecin to 11-nitrocamptothecin, and thereafter converting the 11-nitro group in the resultant compound into various 11-substituents, as in the case of 10-nitrocamptothecin, according to the methods known per se (Japanese Laid-open Patent Appln. No. 59-51987). For example, the 11-nitro group thus formed can first be converted into an 11-amino group by reduction according to Clemensen or the like method and the latter 11-amino group can further be converted into an 11-hydroxy group by diazotization with a nitrite under cooling and acidic conditions followed by hydrolysis under warming and weakly alkaline conditions.

From the studies on various camptothecin derivatives prepared heretofore, the present inventors obtained an interesting result that introduction of an alkyl group into the 7-position of camptothecin tends to enhance anti-tumor activity while introduction of a hydroxyl group into the ring A of camptothecin tends to reduce toxicity without sacrificing the anti-tumor activity. For further extensive researches based on this interesting result for clarifying the relation between the substituents and locations thereof in camptothecin structure and pharmacological properties including toxicity, therefore, there is still a great demand in this art for developing further new classes of camptothecin derivatives carrying various substituents especially in 7-position and on the ring A of camptothecin skeleton.

BRIEF SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide new camptothecin derivatives substituted or unsubstituted in the 7-position thereof and carrying an acyloxy group (more precisely, an aminoacyloxy group) or a chloroacyloxy group in any of the 9-, 10- and 11-positions on the ring A thereof.

It is another object of the present invention to provide new camptothecin derivatives which are strong in anti-tumor activity and possess good solubility in water and an extremely low toxicity.

It is still another object of the present invention to provide a process for the preparation of various camptothecin derivatives carrying an acyloxy group in any of the 9-, 10- and 11-positions thereof according to an easy and economically advantageous operations.

It is a further object of the present invention to provide a new means for introducing an aminocarbonyloxy group or a chlorocarbonyloxy group into any of the 9-, 10- and 11-positions of camptothecin or a 7-substituted derivative thereof.

It is a still further object of the present invention to provide a new means for solubilizing camptothecin or a derivative thereof in water without causing any serious reduction in anti-tumor activity.

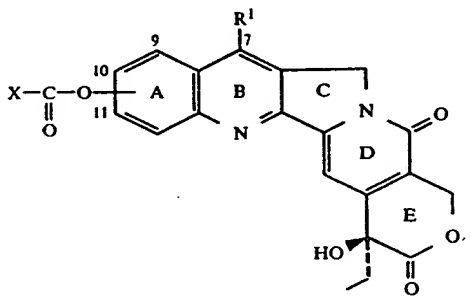
Other objects, features and advantages of the present invention will become apparent more fully from the following description.

DETAILED DESCRIPTION OF THE INVENTION

With an attempt to prepare a new class of camptothecin derivatives which are strong in inherent anti-tumor activity and possess good solubility in water with an extremely low toxicity, the present inventors have made extensive researches for making chemical modifications in the 9-, 10- and 11-positions of camptothecin structure, using camptothecin derivatives already reported or prepared by the present inventors and coworkers which carry a hydroxyl group in any of the 9-, 10- and 11-positions as starting materials, taking careful attention to the chemical modification so that a solubilizing function may be contained in a substituent to be introduced and lest any destroy should occur in the other ring structures, especially in the ring D and/or E. As a result of the extensive researches, it has now been found surprisingly that new camptothecin derivatives of the expected pharmacological properties can be obtained by combining with the hydroxyl group existing in any of the 9-, 10- and 11-positions a chemically stable aminocarbonyl or chlorocarbonyl group which contains a solubilizing function and can be split off enzymatically in vivo. It has also been found incidentally that introduction of such an aminocarbonyl or chlorocarbonyl group into the hydroxyl group in any of the 9-, 10- and 11-positions on the ring A of camptothecin can be achieved by treating a camptothecin derivative carrying a hydroxyl group in any of the 9-, 10- and 11-positions thereof with phosgene and optionally reacting the resultant chlorocarbonyloxy derivative with an amine, or alternatively by treating the camptothecin derivative carrying a hydroxyl group in any of the 9-, 10- and 11-positions thereof directly with a reactive functional derivative of a carbamic acid.

A new class of camptothecin derivatives carrying such aminocarbonyloxy or chlorocarbonyloxy group thus obtained are improved in pharmacological properties and extremely reduced in toxicity. The present invention has been accomplished on the basis of the above finding.

In accordance with the present invention, there are provided new camptothecin derivatives of the general formula:



(I)

wherein R^1 is a hydrogen atom, a halogen atom or an alkyl group with 1–4 carbon atoms and X is a chlorine atom or $—NR^2R^3$ where R^2 and R^3 are the same or different and each represents a hydrogen atom, a substituted or unsubstituted alkyl group with 1–4 carbon atoms or a substituted or unsubstituted carbocyclic or heterocyclic group, with the proviso that when both R^2 and R^3 are the substituted or unsubstituted alkyl groups, they may be combined together with the nitrogen atom, to which R^2 and R^3 are bonded, to form a heterocyclic ring which may be interrupted with —O—, —S— and/or $>N—R^4$ in which R^4 is a hydrogen atom, a substituted or unsubstituted alkyl group with 1–4 carbon atoms or a substituted or unsubstituted phenyl group, and wherein the grouping —O—CO—X is bonded to a carbon atom located in any of the 9-, 10- and 11-positions in the ring A of camptothecin, as well as an ammonium salt or an alkali metal salt thereof.

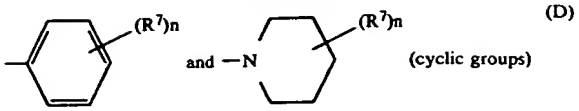
In the general formula (I), the radical R^1 located in 7-position of the ring B is preferably a halogen atom or an alkyl group with 1–4 carbon atoms, with the alkyl group being most preferable. The acyloxy grouping —O—CO—X can be bonded to a carbon atom located in any of the 9-, 10- and 11-positions in the ring A of camptothecin but is preferably bonded to the 10-position thereof.

Examples of R^1 being a halogen atom include fluorine atom, chlorine atom, bromine atom and iodine atom, with the fluorine and chlorine atoms being preferable. Illustrative of R^1, R^2, R^3 or R^4 in case of an alkyl group with 1–4 carbon atoms are straight or branched chain C_{1-4} alkyl groups, i.e. methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl and tert.-butyl groups, with the methyl and ethyl groups being preferable.

When R^2, R^3 or R^4 is an alkyl group with 1–4 carbon atoms as exemplified above, it may be substituted by one or more substituents selected from the following atoms and/or groups:

—F, —Cl, —Br and —I (halogen atoms), (A)
—OH and $—OR^5$ (hydroxy, alkoxy and phenoxy groups), (B)
$—COOR^6$, $—SO_3R^6$ and $—PO_3(R^6)_2$ (acid functions), (C)

-continued



(D)

(cyclic groups)

$—NR^8R^9$ and $—CONR^8R^9$ (amino and amido groups), and (E)
$—Q—A—OR^5$, $—Q—A—NR^8R^9$ and (F)
$—Q—A—Q—R^5$ (ester groups)

wherein R^5 is an alkyl group with 1–4 carbon atoms or a phenyl group which may be substituted by a halogen atom or an alkyl group with 1–4 carbon atoms, R^6 is a hydrogen atom or an alkyl group with 1–4 carbon atoms, R^7 is a hydrogen atom, a halogen atom, an alkyl group with 1–4 carbon atoms or an alkoxy group with 1–4 carbon atoms, n is an integer of 1–3, R^8 and R^9 are the same or different and each represents a hydrogen atom or an alkyl group with 1–4 carbon atoms with the proviso that when both R^8 and R^9 are the alkyl groups, they may be combined together with the nitrogen atom, to which they are bonded, to form a heterocyclic ring which may be interrupted with —O—, —S— or $>N—R^6$, Q is the grouping —O—CO— or —CO—O—, and A is a straight or branched chain alkylene group with 1–4 carbon atoms.

In the case (A), preferable halogen atoms are fluorine and chlorine atoms. Examples of the halogen-substituted alkyl group include fluoromethyl, 2-fluoroethyl, trifluoromethyl, chloromethyl, 1- or 2-chloroethyl, 3-chloro-n-propyl, dichloromethyl, 1,2-dichloroethyl, trichloromethyl, bromomethyl, 1- or 2-bromoethyl, dibromomethyl, 1,2-dibromoethyl, tribromomethyl and iodomethyl groups. When two or more halogen atoms are present in the alkyl group, they may be the same or different such as fluorochloromethyl or 1-chloro-2-bromoethyl.

In the case (B), the alkyl group desirably contains one hydroxyl group. Preferable examples of the hydroxyl-substituted alkyl group include hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl and 3-hydroxypropyl group. When R^5 is an alkyl group with 1–4 carbon atoms, the substituent is an alkoxy group with 1–4 carbon atoms and this case just corresponds to the case (D) wherein R^7 is an alkoxy group with 1–4 carbon atoms. In these cases, the alkyl moiety of the alkoxy group corresponds in principle to the aforesaid alkyl group with 1–4 carbon atoms. Illustrative of such alkoxy group are methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, isobutoxy and tert-butoxy groups. Preferable examples of the alkyl group substituted by such alkoxy group or groups include methoxymethyl, 2-methoxyethyl, 3-methoxypropyl, 4-methoxybutyl, ethoxymethyl, 2-ethoxyethyl, 3-ethoxypropyl, propoxymethyl, 2-propoxyethyl, 3-propoxypropyl, 4-propoxybutyl, dimethoxymethyl, 2,2-dimethoxyethyl, diethoxymethyl, 2,2-diethoxyethyl, dipropoxymethyl and 2,2-dipropoxyethyl groups. In case of R^5 being a substituted or unsubstituted phenyl group, preferable examples of the phenyl-substituted alkyl group include phenoxymethyl, p-methylphenoxymethyl, o-chlorophenoxymethyl, 2-phenoxyethyl, 3-phenoxypropyl and 4-phenoxybutyl group.

In the case (C), the substituents having acid function are usually present in the form of an ester with a lower alkanol with 1–4 carbon atoms, in particular with methanol or ethanol, and in some cases as alkali metal salts or ammonium salts. Thus, R^6 is preferably an alkyl group

with 1–4 carbon atoms as exemplified with respect to R^1–R^4. Illustrative of the alkyl group substituted by such acids or esters thereof are, for example, carboxymethyl, methoxycarbonylmethyl, ethoxycarbonylmethyl, 2-ethoxycarbonylethyl, ethoxysulfonylmethyl and diethoxyphosphonylmethyl groups. The substituents in the form of esters can easily be saponified, if desired, to the free carboxylic, sulfonic and phosphonic acid groups or the alkali metal salts thereof.

In the case (D), the phenyl group is preferably substituted by one substituent R^7 in any desired position but may be substituted by two or three substituents R^7 which may be the same or different. When two substituents R^7 are present in the phenyl group, they are preferably present in 2- and 4-positions or 2- and 6-positions. When three substituents R^7 are present in the phenyl group, they are preferably located in 2-, 4- and 6-positions. When R^7 is a halogen atom, the phenyl group preferably has one fluorine, chlorine or bromine atom but may have up to 3 halogen atoms as mentioned above. When R^7 is an alkyl or alkoxy group with 1–4 carbon atoms, the alkyl moiety of such group just corresponds to the alkyl group as exemplified with respect to R^1–R^4. Illustrative of the phenyl-substituted alkyl group are, for example, benzyl, phenethyl, 3-phenylpropyl, 4-phenylbutyl, 2-fluorobenzyl, 2-chlorobenzyl, 4-ethylbenzyl, 4-n-propylbenzyl, 4-methoxybenzyl, 4-fluorophenethyl, 4-methoxyphenethyl, 2,4-dichlorobenzyl, 2,4-dimethylbenzyl, 2,4-dimethoxybenzyl, 2,4,6-trichlorobenzyl, 2,4-dichlorophenethyl, 3-(2,4-dimethoxyphenyl)propyl, 3-(4-ethylphenyl)propyl, 3-(4-propoxyphenyl)propyl and 3-(2,4-dibutoxyphenyl)propyl groups.

In case of R^2 or R^3 being an alkyl group carrying a piperidyl group as a substituent thereof, the piperidyl group is present as a rule in the ω-position of the alkyl group. Typical examples of such piperidyl-(1)-alkyl group include 1-piperidylmethyl, 2-(1-piperidyl)ethyl, 3-(1-piperidyl)propyl and 4-(1-piperidyl)butyl groups. The piperidyl group is preferably not substituted (R^7=H) but may be substituted with 1–3 alkyl, alkoxy and/or halogen substituents ($R^7 \neq H$) in the same manner as in the case of the phenyl-substituted alkyl groups.

In the case (E), at least one of the groups R^8 and R^9 as well as R^6 is preferably an alkyl group with 1–4 carbon atoms as exemplified with respect to R^1–R^4. Preferable examples of the grouping NR^8R^9 and of the amino moiety NR^8R^9 in the grouping $CONR^8R^9$ include amino, N-methylamino, N-ethylamino, N-propylamino, N-butylamino, N,N-dimethylamino, N,N-diethylamino, N-methyl-N-ethylamino, N,N-dipropylamino, N,N-dibutylamino and heterocyclic groups such as 1-piperidino, 4-morpholino and 4-methyl-1-piperazino groups.

In the case (F), the substituent groupings can be regarded as ester components. In case of —Q—A—OR^5 wherein Q is —CO—O—, an alkylenediol —A—OR^5 is linked with the alkyl group through the carboxyl function. In case of —Q—A—OR^5 wherein Q is —O—CO—, a hydroxy acid is linked with a hydroxy-substituted alkyl group in the case (B). In case of —Q—A—NR^8R^9 wherein Q is —O—CO—, the alkyl group substituted by hydroxyl group in the case (B) is esterified with an amino acid. In case of —Q—A—Q—R^5 wherein Q—A—Q is —O—CO—A—CO—O—, the alkyl group sustituted by hydroxyl group in the case (B) is linked with a dicarboxylic acid or a hemiester thereof. The group A is derived in principle from the alkyl groups as exemplified above and is preferably methylene, ethylene or propylene-1,3. Examples of the alkyl group substituted by such ester components include methoxyethoxycarbonylethyl, hydroxymethylcarbonyloxymethyl, glycyloxymethyl, dimethylaminoethylcarbonyloxymethyl and methoxycarbonylethylcarbonyloxymethyl (methoxysuccinyloxymethyl) group.

In case R^2 or R^3 is a carbocyclic or heterocyclic group which may be substituted, such cyclic ring is preferably saturated but may be of aromatic nature and is usually selected from cycloalkyl, phenyl and saturated or unsaturated aromatic heterocyclic groups. As substituents on such cyclic group, a halogen atom such as fluorine, chlorine or bromine atom, an alkyl group with 1–4 carbon atoms and an alkoxy group with 1–4 carbon atoms are useful. Examples of the saturated carbocyclic and heterocyclic groups include cyclopentyl, cyclohexyl, N-methyl-piperidyl-(4) and 2-pyrrolidyl groups. Examples of the carbocyclic and heterocyclic groups of aromatic nature include phenyl, tolyl, xylyl, pyridyl-2 and 2-methylpyridyl-(4) groups.

When alkyl groups R^2 and R^3 are combined to form a heterocyclic ring together with the nitrogen atom to which they are bonded, such nitrogen-containing heterocyclic group is selected from 5-membered and 6-membered saturated heterocyclic groups such as pyrrolidino and piperidino groups. This heterocyclic ring system may be interrupted with —O—, —S— or

to form, for example, 2-oxapyrrolidino, morpholino, thiomorpholino, piperazino and 4-C_{1-4} alkylpiperazino groups. Preferable heterocyclic groups are pyrrolidino, piperidino, morpholino, piperazino and 4-C_{1-4} alkylpiperazino groups. If R^4 on the 4-nitrogen atom of the piperazino ring is a substituted alkyl group, it may carry a substituent or substituents selected from the atoms and/or groups shown in the cases (A)–(F). Examples of such substituted alkyl group R^4 include 2-bromoethyl, ethoxymethyl, methoxycarbonylmethyl, 2-ethylbenzyl, 2-(1-piperidyl)ethyl, N-methylaminomethyl, N,N-diethylaminomethyl, N-isopropylcarbamoylmethyl, 2-ethoxy-ethoxycarbonylmethyl, N,N-dimethylaminomethoxycarbonylmethyl and 4-ethoxycarbonylbutylcarbonyloxymethyl groups.

When either or both of the alkyl groups R^2 and R^3 are substituted by a substituent or substituents selected from the atoms and/or groups shown in the cases (A)–(F) and are combined to form such heterocyclic ring together with the nitrogen atom to which R^2 and R^3 are bonded, the substituent or substituents in the alkyl groups R^2 and/or R^3 will form a ring substituent or substituents in the resultant heterocyclic group and/or a side chain or chains extending from the alkyl main chain on the heterocyclic ring. For example, 3-(1-piperidyl)-propyl as R^2 and ethyl as R^3 are combined together with the nitrogen atom, to which they are bonded, to form 4-[(1-piperidino)-1-piperidino]. Likewise, 3-(1-piperidyl)propyl as R^2 and 1-chloroethyl as R^3 are combined together with the nitrogen atom, to which they are bonded, to form 4-[1-piperidyl)-1-(2-chloro)-piperidino]. In case 3-dimethylaminopropyl as R^2 and 1-chloroethyl as R^3 are combined together with the

nitrogen atom to which they are bonded, a 2-chloro-4-dimethylamino-1-piperidino group is formed.

In case R^2 and/or R^3 is an amino-substituted alkyl group in the case (E) where R^8 and R^9 are hydrogen atoms, a water-soluble ammonium salt can be formed at the nitrogen atom of the amino group with an inorganic or organic acid. In general, inorganic acids and organic acids capable of forming a water-soluble salt with a camptothecin derivative having an amino group are physiologically acceptable and are selected, for example, from hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, nitric acid, acetic acid, propionic acid, l-ascorbic acid, tartaric acid, citric acid, lactic acid, maleic acid, fumaric acid, methanesulfonic acid, toluenesulfonic acid and benzenesulfonic acid.

On the other hand, when R^2 and/or R^3 is an alkyl group substituted by an acid function in the case (C) such as carboxylic acid, sulfonic acid or phosphonic acid group or an ester form thereof, the acid function can be converted into an alkali metal salt form by the treatment with an alkali metal hydroxide or carbonate such as sodium hydroxide or carbonate, or potassium hydroxide or carbonate.

The E-ring structure of the camptothecin derivatives of this invention is not damaged by the formation of the above water-soluble salts. Thus, the pharmacological activities of the camptothecin derivatives are never affected by conversion to their water-soluble salts. It is believed that the camptothecin derivatives are easily converted to their 9-, 10- or 11-hydroxy (free) form in vivo by the action of a carboxyamidase or the like enzyme.

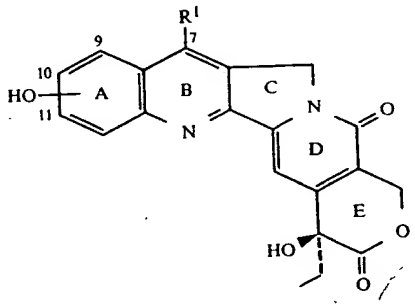
The 9-, 10- or 11-substituted new camptothecin derivatives of the present invention represented by the general formula (I) possess strong anti-tumor activity with reduced toxicity. Illustrative of the new camptothecin derivatives of the present invention are, for example, 9-chlorocarbonyloxycamptothecin (9-chlorocarbonyloxy-CPT; "camptothecin" will be referred to hereinafter simply as "CPT"), 9-chlorocarbonyloxy-7-ethyl-CPT, 10-chlorocarbonyloxy-CPT, 10-chlorocarbonyloxy-7-ethyl-CPT, 11-chlorocarbonyloxy-CPT, 11-chlorocarbonyloxy-7-ethyl-CPT, 7-ethyl-9-[4-(N-isopropylcarbamoylmethyl)-1-piperazino]carbonyloxy-CPT, 9-(1-piperazino)carbonyloxy-CPT, 9-(4-methyl-1-piperazino)carbonyloxy-CPT, 9-[4-(N-isopropylcarbamoylmethyl)-1-piperazino]carbonyloxy-CPT, 9-[4-(1-piperidino)-1-piperidino]carbonyloxy-CPT, 9-[N-methyl-N-(2-dimethylaminoethyl)]carbonyloxy-CPT, 7-ethyl-9-(1-piperazino)carbonyloxy-CPT, 7-ethyl-9-(4-methyl-1-piperazino)carbonyloxy-CPT, 7-ethyl-9-[4-(N-isopropylcarbamoylmethyl)-1-piperazino]carbonyloxy-CPT, 7-ethyl-9[4-(1-piperidino)-1-piperidino]carbonyloxy-CPT, 7-ethyl-9-[N-propyl-N-(2-dimethylaminoethyl)]carbonyloxy-CPT, 9-(1-piperazino)carbonyloxy-7-propyl-CPT, 10-[(N-ethoxycarbonylmethylamino)carbonyloxy]-7-ethyl-CPT, 10-(2-diethylamino)-ethyl-aminocarbonyloxy-7-ethyl-CPT, 10-diethylaminocarbonyloxy-7-ethyl-CPT, 7-ethyl-10-(4-morpholino)carbonyloxy-CPT, 7-ethyl-10-(1-piperazino)carbonyloxy-CPT, 7-ethyl-10-(4-methyl-1-piperazino)carbonyloxy-CPT, 7-ethyl-10-(4-ethyl-1-piperazino)carbonyloxy-CPT, 10-(4-benzyl-1-piperazino)carbonyloxy-7-ethyl-CPT, 7-ethyl-10-[4-(p-methoxyphenyl)-1-piperazino]carbonyloxy-CPT, 7-ethyl-10-[4-(3-hydroxypropyl)-1-piperazino]carbonyloxy-CPT, 7-ethyl-10-[4-(N-isopropylcarbamoylmethyl)-1-piperazino]carbonyloxy-CPT, 7-ethyl-10-[4-(1-piperidino)piperidino]carbonyloxy-CPT, 7-ethyl-10-[N-methyl-N-(2-dimethylaminoethyl)]aminocarbonyloxy-CPT, 7-ethyl-10-N-methyl-N-(1-methyl-4-piperidino)aminocarbonyloxy-CPT, 10-(4-morpholino)-carbonyloxy-CPT, 10-(4-methyl-1-piperazino)carbonyloxy-CPT, 7-ethyl-10-(4-propyl-1-piperazino)carbonyloxy-CPT, 7-ethyl-10-(4-methyl-1-piperazino)carbonyloxy-CPT, 11-(4-ethyl-1-piperazino)carbonyloxy-CPT, 11-[4-(1-piperidino)-1-piperidino]carbonyloxy-CPT, 11-(1-piperazino)carbonyloxy-CPT, 11-(4-methyl-1-piperazino)carbonyloxy-CPT, 11-[4-(N-isopropylcarbamoylmethyl)-1-piperazino]carbonyloxy-CPT, 11-[N-methyl-N-(2-dimethylaminoethyl)]carbonyloxy-CPT, 7-ethyl-11-(1-piperazino)carbonyloxy-CPT, 7-ethyl-11-(4-methyl-1-piperazino)carbonyloxy-CPT, 7-ethyl-11-[4-(N-isopropylcarbamoylmethyl)-1-piperazino]carbonyloxy-CPT, 7-ethyl-11-[N-methyl-N-(2-dimethylaminoethyl)]carbonyloxy-CPT and 7-ethyl-11-[4-(1-piperidino)-1-piperidino]carbonyloxy-CPT.

In accordance with the present invention, there is also provided a process for the preparation of the 9-, 10- or 11-substituted new camptothecin derivatives of the general formula (I).

The process of this invention wherein 9-, 10- or 11-hydroxy-7-R^1-camptothecin is used as starting material comprises two embodiments; one being directed to the reaction with phosgene optionally followed by amination with an amine to prepare both 9-, 10- or 11-chlorocarbonyloxy or aminocarbonyloxy compounds and the other being directed to the reaction with a carbamoyl chloride to exclusively obtain the corresponding 9-, 10- or 11-aminocarbonyloxy compounds.

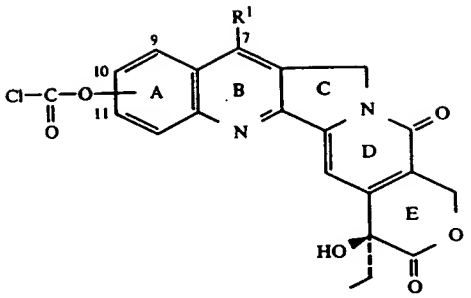
In one embodiment of the process, the new camptothecin derivatives of the general formula (I) as well as ammonium salts or alkali metal salts thereof can be prepared by reacting a hydroxycamptothecin derivative of the general formula:

(II)



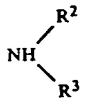
wherein R^1 is a hydrogen atom, a halogen atom or an alkyl group with 1–4 carbon atoms and the hydroxy group OH is bonded to a carbon atom located in any of the 9-, 10- and 11-positions in the ring A of camptothecin, with phosgene to form a chlorocarbonyloxycamptothecin derivative of the general formula:

(III)

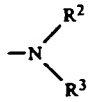


wherein R^1 has the same meaning as given above and the grouping Cl—CO—O— is bonded to a carbon atom located in any of the 9-, 10- and 11- positions in the ring A of camptothecin, and if necessary, treating the chlorocarbonyloxycamptothecin derivative (III) with an amine of the general formula:

(IV)



wherein R^2 and R^3 are the same or different and each represents a hydrogen atom, a substituted or unsubstituted alkyl group with 1–4 carbon atoms or a substituted or unsubstituted carbocyclic or heterocyclic group with the proviso that when both R^2 and R^3 are the substituted or unsubstituted alkyl groups, they may be combined together with the nitrogen atom, to which R^2 and R^3 are bonded, to form a heterocyclic ring which may be interrupted with —O—, —S— and >N—R^4 in which R^4 is a hydrogen atom, a substituted or unsubstituted alkyl group with 1–4 carbon atoms or a substituted or unsubstituted phenyl group, and if desired, converting R^2 and/or R^3 in the resultant aminocarbonyloxycamptothecin derivative of the general formula (I) where X is



into another R^2 and /or R^3 by N-alkylation or O-alkylation according to the method known per se and/or converting the resultant aminocarbonyloxycamptothecin derivative into an ammonium salt thereof with an acid or into an alkali metal salt thereof with an alkali metal base.

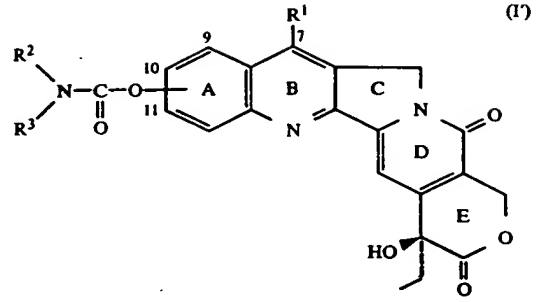
The hydroxycamptothecin derivatives of the general formula (II) used as the starting material are known or can be prepared according to the known prior art processes. In case of 9-hydroxycamptothecin derivatives, i.e. the hydroxycamptothecin derivatives of the general formula (II) wherein the hydroxyl group —OH is located in the 9-position camptothecin is first treated carefully with nitric acid under ice-cooling to introduce a nitro group into the 9-position of camptothecin and the resultant 9-nitrocamptothecin is then treated with a reducing agent such as a combination of tin or iron with a mineral acid or is hydrogenated catalytically to form 9-aminocamptothecin. This 9-amino derivative is then treated in an acidic solution with a nitrite to form the corresponding diazonium compound which is then hydrolyzed to 9-hydroxycamptothecin (Japanese Laid-open Patent Appln. No. 59-51289). If necessary, this hydroxy derivative is alkylated in the 7-position (U.S. Pat. No. 4,399,282) or halogenated in the 7-position via N-oxidation. In case of 10-hydroxycamptothecin derivatives, i.e. the hydroxycamptothecin derivatives of the general formula (II) wherein the hydroxyl group —OH is located in the 10-position, a 7-R^1-camptothecin is treated in a liquid vehicle such as acetic acid with a peroxide to form the corresponding N-oxide which is then irradiated with actinic light in the presence of a solvent or a solvent mixture selected from methyl cellosolve, dioxane, acetonitrile, chloroform, methylene chloride, glyme and diglyme and in the presence of a mineral acid such as sulfuric acid or perchloric acid or an organic sulfonic acid (Japanese Laid-open Patent Appln. No. 58-39685; U.S. Ser. No. 414,528). In case of 11-hydroxycamptothecin derivatives, i.e. the hydroxycamptothecin derivatives of the general formula (II) wherein the hydroxyl group —OH is located in 11-position, camptothecin is subjected to catalytic hydrogenation to form 1,2,6,7-tetrahydrocamptothecin (the ring-B-hydrogenated derivative) which is then subjected to a series of reactions, i.e. nitration and simultaneous dehydrogenation in the presence of concentrated sulfuric acid, reduction of the resultant 11-nitrocamptothecin to the corresponding 11-amino derivative, the subsequent diazotization to the corresponding diazonium salt, and hydrolysis of the diazonium salt by warming (Japanese Laid-open Patent Appln. No. 59-51287). If necessary, the resultant 11-hydroxycamptothecin is subjected to 7-alkylation or to 7-halogenation.

Primary and secondary amines of the general formula (IV) are known and easily commercially available. When R^2, R^3 and R^4 are substituted alkyl groups, the substituents are selected from the aforesaid cases (A)–(F). When the phenyl group is substituted, the substituent or substituents correspond to R^7 in the case (D). The carbocyclic and heterocyclic groups are selected usually from cycloalkyl, phenyl and saturated or aromatic heterocyclic groups and the substituents are selected from halogen atoms, alkyl groups with 1–4 carbon atoms and alkoxy groups with 1–4 carbon atoms. Accordingly, a wide variety of amines can be used as the reactant HNR^2R^3. Illustrative of the amine are, for example, ammonia, a primary C_{1-4} alkylamine such as methylamine or ethylamine, a secondary C_{1-4} dialkylamine such as dimethylamine or diethylamine, a halogen-substituted primary or secondary alkylamine such as 2-chloroethylamine, a hydroxy- or alkoxy-substituted amine such as 2-hydroxyethylamine or 2,2'-dimethoxydiethylamine, an amine having an acid function such as ethoxycarbonylmethylamine or ethoxysulfonylethylamine, a phenylalkylamine such as benzylamine, 4-methoxyphenylpropylamine or 2,4-dichlorophenethylamine, an aminoalkylamine such as 2-(N,N-diethylamino)ethylamine, an aminocarbonylalkylamine such as isopropylaminocarbonylethylamine or dimethylaminoethylmethylamine, a heterocyclic amine such as morpholine, piperidine, piperazine, N-benzylpiperazine, 1-methylpiperazine, 4-(1-piperidino)piperidine or 4-(4-methoxyphenyl)piperazine, an esterified alkylamine such as methoxyethoxycarbonylmethylamine, dimethylaminoethoxycarbonylmethylamine or methoxycarbonylethylcarbonyloxypropylamine, and other cyclic amine such as N-methyl-4-piperidylmethylamine.

In the first step of the process, a hydroxycamptothecin of the general formula (II) is dissolved in an anhydrous solvent and gaseous phosgene is introduced into the solution under agitation at a temperature in a wide range, preferably at room temperature. Preferable examples of the solvent for the hydroxycamptothecin include dioxane, acetone, chloroform, methylene chloride, methanol, ethanol and a mixture of these solvents. The gaseous phosgene is usually employed in a slightly excess amount. The condensation reaction of the hydroxycamptothecin with phosgene proceeds while splitting off hydrogen chloride. Thus, the reaction can be promoted rapidly by addition of an acid-binding agent to the reaction system. A tertiary amine such as triethylamine, pyridine, picoline, 1,8-diazabicyclo[5.4.0]undec-7-en (DBU), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) or trimethylamine, an alkali metal hydride such as sodium hydride, and an alcoholate such as sodium ethoxide or potassium tert-butoxide are suitable acid-binding agents in this case and are usually employed in a slightly excess amount. The end point of the reaction is confirmed by complete consumption of the starting material. For this purpose, a trace amount of the reaction mixture is sampled and subjected to TLC or the like analysis to detect the starting material. The reaction is finished usually within one hour. After completion of the reaction, any insoluble matter is removed by filtration and the filtrate is allowed to evaporate under reduced pressure until dryness whereby a chlorocarbonyloxycamptothecin of the general formula (III) is obtained quantitatively as a light yellowish white powdery substance.

In the second step of the process, a chlorocarbonyloxycamptothecin of the general formula (III) is suspended in a solvent and an amine of the general formula (IV) is then added to the suspension under agitation. The reaction is conducted under warming or at room temperature. The solvent used in this second step is usually identical with that used in the first step. This amination reaction proceeds with the liberation of hydrogen chloride. Thus, the reaction can be promoted by using an acid-binding agent as in the first step. In this amination reaction, therefore, the amine is used in an excess amount, a part of which functions as an acid-binding agent to capture the liberated hydrogen chloride thereby promoting the reaction. It is a matter of course that a tertiary amine or a metal compound as exemplified in the first step can be used as an acid-binding agent. In this case, the amount of the amine to be reacted with the compound of the general formula (III) can be reduced. A part of the reaction mixture is occasionally sampled and analyzed to confirm whether the starting material has entirely been consumed or not. The reaction is finished usually within 36 hours at room temperature but the reaction time can be shortened by warming the reaction mixture to accelerate the reaction. After completion of the reaction, the solvent is distilled off under reduced pressure and the residue is then extracted with a solvent such as dioxane. The solvent is removed from the extract and the residue is then subjected to separation and purification by way of column chromatography or T.L.C. on silica gel. Various aminocarbonyloxycamptothecin derivatives (X is $-NR^2R^3$ is in the general formula I) can thus be obtained.

In another embodiment of the process of this invention, such aminocarbonyloxycamptothecin derivatives of the general formula:



(I')

wherein R^1, R^2 and R^3 have the same meanings as given above and wherein the grouping $R^2R^3N—CO—O—$ is bonded to a carbon atom in any of the 9-, 10- and 11-positions thereof, as well as ammonium salts or alkali metal salts thereof, can be prepared by reacting a hydroxycamptothecin of the general formula (II) with a carbamoyl chloride of the general formula (V):

$$Cl—CO—NR^2R^3 \quad (V)$$

wherein R^2 and R^3 have the same meanings as given above, in a liquid vehicle preferably in the presence of an acid-binding agent, and if desired, converting R^2 and/or R^3 in the resultant aminocarbonyloxycamptothecin derivative of the general formula (I') into another R^2 and/or R^3 by N-alkylation or O-alkylation according to the method known per se and/or converting the resultant aminocarbonyloxycamptothecin derivative into an ammonium salt thereof with an acid or into an alkali metal salt thereof with an alkali metal base.

Carbamoyl chlorides of the general formula (V) can be prepared in a high purity and in a high yield according to the method known per se by reacting an amine of the general formula (IV) in a solvent with phosgen or phosgen dimer. Examples of the solvent used in this case include non-polar inert solvents such as benzene, toluene or the like aromatic hydrocarbon and hexane or the like aliphatic hydrocarbons.

The reaction between the hydroxycamptothecin derivative of the general formula (II) and the carbamoyl chloride of the general formula (V) is carried out by usually using the latter in an excess amount (1.2–2.0 times as much as the theoretical amount) at room temperature or under warming. When the carbamoyl chloride is used in an amount more than 1.8 times of the theoretical amount, the reaction will be finished within a short period of time at room temperature. Illustrative of the liquid vehicle used for this reaction are, for example, polar aprotic solvents such as dimethylformamide, diethylformamide, dimethylsulfoxide, hexamethylphosphoramide and pyridine. Preferable examples of the acid-binding agent include a metal hydride such as sodium hydride, a metal alcoholate such as potassium tert-butoxide, and a tertiary amine such as triethylamine, 4-(N,N-dimethylamino)pyridine, pyridine, picoline, lutidine, collidine, DBU and DBN. The use of pyridine is preferable because it is good for dual purposes of solvent and acid-binding agent. These tertiary amines may be used in an excess amount for dual purpose as the solvent and the acid-binding agent. The reaction is usually finished within 20 hours and within 2 hours in a preferable case.

The aminocarbonyloxycamptothecin derivatives of this invention thus obtained can further be treated with

a reagent for converting R^2 and/or R^3 into another R^2 and/or R^3. In case R^2 and/or R^3 is a hydrogen atom or an alkyl group carrying as substituent OH or $—NR^8R^9$ where at least one of R^8 and R^9 is a hydrogen atom or in case R^2 and R^3 are combined together with the nitrogen atom, to which they are bonded, to form a heterocyclic ring interrupted with $>N—R^4$ where R^4 is a hydrogen atom, such hydrogen atom can be converted into an alkyl group by N- or O-alkylation according to the method known per se for N-alkylation or O-alkylation. An alkyl halide with 1–4 carbon atoms such as methyl iodide, ethyl bromide or propyl bromide, a dialkyl sulfate with 1–4 carbon atoms in each alkyl moiety such as dimethyl sulfate or diethylsulfate, a diazoalkane with 1–4 carbon atoms such as diazomethane and the like alkylating agents come into question as the alkylating agents for this purpose. For example, 10-(2-aminoethylmethyl)aminocarbonyloxycamptothecin can be treated with ethyl bromide to form the corresponding 10-(2-diethylaminoethylmethyl)aminocarbonyloxy derivative and 10-piperazinocarbonyloxycamptothecin can be treated with propyl bromide to form the corresponding 10-(4-propyl-1-piperazino)carbonyloxy derivative.

The new camptothecin derivative of the general formula (I) wherein R^2 and/or R^3 has an amino group or of the general formula (I') can be treated, if desired, with a stoichiometrical amount of an acid such as hydrochloric acid, p-toluenesulfonic acid, acetic acid, tartaric acid, maleic acid or fumaric acid to form a water-soluble ammonium salt of the camptothecin derivative. On the other hand, the camptothecin derivative wherein R^2 and/or R^3 has an acid function such as carboxyl group or an alkoxycarbonyl group can be treated, if desired, with a stoichiometrical amount of a strong alkali metal base such as sodium hydroxide, potassium hydroxide or the like to form a water-soluble alkali metal salt.

The water-soluble ammonium salts or alkali metal carboxylates can be converted, if necessary, into the free form by treating the salts with an alkaline substance such as sodium hydroxide or sodium carbonate or treating the carboxylates with a strong or weak acid such as hydrochloric acid or acetic acid, respectively.

The process starting with the compound of the general formula (II) is convenient for preparing a wide variety of the aminocarbonyloxycamptothecin derivatives, while the process starting with the compound of the general formula (V) is convient for preparing a particular end product in a large amount.

The chlorocarbonyloxy- and aminocarbonyloxycamptothecin derivatives of the present invention represented by the general formula (I) can be used as such or after further purification as active ingredients for anti-tumor medicaments or as valuable intermediate products for preparing other useful products. Accordingly, the present invention is of particular significance in developing 9-, 10- or 11-substituted camptothecin derivatives as a new class of camptothecin derivatives useful as anti-tumor medicaments possessing strong anti-tumor activity with reduced toxicity and also as intermediate products for preparing other useful new products as well as a new process for preparing these valuable camptothecin derivatives in a simple economically advantageous operation.

The present invention will now be illustrated in more detail by way of examples.

EXAMPLE 1

9-Chlorocarbonyloxy-7-propylcamptothecin

A suspension of 9-hydroxy-7-propylcamptothecin (100 mg, 0.246 mmol) in dry dioxane (150 ml) containing 400 μl of trimethylamine was warmed gently until the hydroxylic compound was dissolved in the dioxane. After cooling the solution to room temperature, phosgene gas freshly prepared by decomposition of trichloromethoxy chloroformate (100 μl) on charcoal was passed through the solution with stirring, and then the mixture was stirred for 30 min. at room temperature. After confirming that the starting material had disappeared completely by way of TLC (10% MeOH-$CHCl_3$, 365 nm), the precipitated material was filtered off by suction and the filtrate was evaporated to dryness in vacuo. The residual colorless powder was washed with a small amount of dry dioxane, filtered, and then dried in vacuo. The title compound was obtained in 94% yield (108 mg) as colorless powder.

IR νKBr/max cm^{-1}: 2970, 2920, 1770, 1655, 1590, 1500, 1220, 1170.

EXAMPLE 2

9-[4-(Isopropylcarbamoylmethyl)-1-piperazino]carbonyloxycamptothecin

9-Hydroxycamptothecin (190 mg, 0.521 mmol) and 1-chlorocarbonyl-4-(isopropylcarbamoylmethyl)piperazine (257 mg, 1.04 mmol) were dissolved in anhydrous pyridine (12 ml) and the mixture was stirred for 4.5 hours at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residue was dissolved in $CHCl_3$ (100 ml). The $CHCl_3$ solution was shaken with a 7% aqueous solution of $NaHCO_3$ (100 ml), washed with a saturated aqueous solution of NaCl, dried with $MgSO_4$, filtered, and then evaporated to dryness in vacuo. The residual material was purified through silica gel column chromatography with 2% MeOH-$CHCl_3$ as an eluent to give 290 mg (96.9% yield) of the title compound, which was then recrystallized from ethanol to give 160 mg of pale yellow needles.

^{1}H-NMR (DMSO-d_6) δppm: 0.84 (3H, t, J=7 Hz), 1.10 (6H, d, J=6 Hz), 2.84 (2H, q, J=7 Hz), 2.45–2.80 (5H, m), 3.04 (2H, s), 3.40–4.00 (4H, br), 5.32 (2H, s), 6.50 (1H, s, D_2O-exchangeable), 7.40–8.10 (4H, m), 8.56 (1H, s).

EXAMPLE 3

7-Methyl-9-[4-(isopropylcarbamoylmethyl)-1-piperazino]carbonyloxycamptothecin

To a solution of 9-hydroxy-7-methylcamptothecin (100 mg, 0.264 mmol) in pyridine (5 ml) was added 1-chlorocarbonyl-4-(isopropylcarbamoylmethyl)piperazine (120 mg, 0.5 mmol). The mixture was stirred for 18 hours at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residual material was shaken with a mixture of $CHCl_3$ (300 ml) and a 7% aqueous solution of $NaHCO_3$ (300 ml). The $CHCl_3$ phase was separated, washed with a saturated aqueous solution of NaCl (300 ml), dried with anhydrous $MgSO_4$, filtered, and then evaporated to dryness in vacuo. The residual material was purified through silica gel column chromatography with 2%-MeOH-$CHCl_3$ as an eluent to give a pale yellow mass, which was then recrystallized from ethanol whereby 93 mg

(65% in yield) of the title compound was obtained as pale yellow needles.

EXAMPLE 4

7-Ethyl-9-[4-(1-piperidino)piperidino]carbonyloxycamptothecin

(A) 9-Methoxycamptothecin (300 mg, 0.79 mmol) was suspended in water (6 ml) Conc. H_2SO_4 (3 ml) was added dropwise to the suspension until the methoxycamptothecin was dissolved therein. After cooling the solution in an ice-bath, propionaldehyde (0.13 ml, 1.6 mmol) and $FeSO_4.7H_2O$ (60 mg, 0.215 mmol) were added to the solution, and 30% H_2O_2 (0.35 ml, 2.77 mmol) was then added dropwise to the mixture with stirring under cooling in an ice-bath. After addition of the hydrogen peroxide, the reaction mixture was continuously stirred for 30 minutes at room temperature. The resulting mixture was poured into ice-water (1 l), and the precipitate in the solution was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried with $MgSO_4$, filtered, and then evaporated to dryness in vacuo. The residue was recrystallized from EtOH to give 256 mg (79.8% in yield) of 7-ethyl-9-methoxycamptothecin as pale yellow needles.

M.P. 274°-276° C. (dec.) [EtOH].

^{1}H-NMR (in $CDCl_3$) δppm: 1.02 (3H, t, J=8 Hz), 1.33 (3H, t, J=7 Hz), 1.92 (2H, q, J=8 Hz), 3.06-3.77 (2H, m), 4.03 (3H,s), 5.07 (2H,s), 5.28 (1H, d, J=17 Hz), 5.67 (1H, d, J=17 Hz), 6.87-7.10 (1H, m), 7.20-7.83 (3H, m).

MS m/e: 406 [M+].

Elementary analysis as $C_{23}H_{16}N_2O_5$: Calcd. C, 67.97; H, 5.46; N, 6.89; Found C, 67.79; H, 5.38; N, 6.82.

(B) The 7-ethyl-9-methoxycamptothecin (250 mg, 0.62 mmol) thus obtained was dissolved in 47% HBr (5 ml) and the solution was heated at 140° C. for 8 hours with stirring. The mixture was poured into ice-water (1 l) and the precipitated material was collected on a filter by suction. The solid material collected was recrystallized from EtOH whereby 100 mg (44% in yield) of 7-ethyl-9-hydroxycamptothecin was obtained as pale yellow needles.

M.P. 270°-272° C. (dec.) [EtOH].

MS m/e: 392 [M+].

Elementary analysis as $C_{22}H_{20}N_2O_5$: Calcd. C, 67.33; H, 5.14; N, 7.14; Found C, 67.13; H, 5.10; N, 7.33.

(C) 7-Ethyl-9-hydroxycamptothecin (100 mg, 0.27 mmol) obtained in (B) was dissolved in dry pyridine (6 ml). To the solution was added 1-chlorocarbonyloxy-4-piperidinopiperidine (200 mg, 0.87 mmol), and the mixture was stirred for one hour at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residue was purified by way of column chromatography on silica gel with 2%-MeOH-$CHCl_3$ as an eluent whereby the title compound was obtained as a pale yellow solid, which was then recrystallized from EtOH to give 80 mg (50% in yield) of the title compound as pale yellow needles.

M.P. 210°-212° C. (dec.) [EtOH].

^{1}H-NMR (in $CDCl_3$) δppm: 1.00 (3H, t, J=8 Hz), 1.17-2.20 (15H, m), 2.20-2.77 (5H, m), 2.77-3.30 (4H, m), 4.20-4.67 (2H, br), 5.20 (2H, s), 5.22 (1H, d, 16 Hz), 5.70 (1H, d, J=16 Hz), 7.40-7.62 (1H, m), 7.62-8.10 (4H, m).

Elementary analysis as $C_{33}H_{38}N_4O_6 \cdot H_2O$: Calcd. C, 65.54; H, 6.67; N, 9.27; Found C, 65.34; H, 6.50; N, 9.50.

EXAMPLE 5

9-(1-piperazino)carbonyloxy-7-propylcamptothecin (A) 9-Methoxycamptothecin (1.00 g, 2.65 mmol) was suspended in water (20 ml). To the solution, Conc. H_2SO_4 (ca 10 ml) was added until the methoxycamptothecin was dissolved in the mixture. After cooling the solution in an ice-bath, butyrylaldehyde (0.5 ml, 5.2 mmol) and $FeSO_4.7H_2O$ (200 mg, 0.7 mmol) were added to the solution, and 30% H_2O_2 (1.25 ml, 9.3 mmol) was then added dropwise to the mixture under agitation and cooling in an ice-bath. After additional agitation for 12 hours at room temperature, the reaction mixture was poured into ice-water (1 l) and the precipitated formed in the solution was extracted with $CHCl_3$. The $CHCl_3$ layer was separated, washed with water, dried with $MgSO_4$, filtered, and then evaporated to dryness in vacuo. The residue was recrystallized from EtOH whereby 520 mg (46.7% in yield) of 9-methoxy-7-propylcamptothecin was obtained as pale yellow needles.

M.P. 276°-278° C. (dec.) [EtOH].

MS m/e: 420 [M+].

Elementary analysis as $C_{24}H_{24}N_2O_5$: Calcd. C, 68.56; H, 5.75; N, 6.66; Found C, 68.46; H, 5.70; N, 6.80.

9-Methoxy-7-propylcamptothecin (500 mg, 1.2 mmol) was dissolved in 47% HBr (5 ml) and the solution was heated at 140° C. for 8 hours with stirring. The mixture was poured into ice-water (1 l) and the precipitated material was collected on a filter by suction and the collected material was recrystallized from EtOH to give 200 mg (41.1% in yield) of 9-hydroxy-7-propylcamptothecin as pale yellow needles.

M.P. 280° C.

Elementary analysis as $C_{23}H_{22}N_2O_5$: Calcd. C, 67.96; H, 5.46; N, 6.89; Found C, 67.77; H, 5.30; N, 6.99.

(B) 9-Hydroxy-7-propylcamptothecin (220 mg, 0.54 mmol) is dissolved in dry dioxane (500 ml) containing 0.3 ml of triethylamine. Phosgen gas (phosgen dimer 0.1 ml, 1.6 mmol) is introduced into the solution under stirring at room temperature. After additional stirring for 3 hours at room temperature, the precipitated material is removed by filtration and the filtrate is evaporated to dryness in vacuo. The residue (9-chlorocarbonyloxy-7-propylcamptothecin) is dissolved in 20% methanol-chloroform (100 ml) containing 0.3 ml of triethylamine. To the mixture is added anhydrous piperazine (51 mg, 0.65 mmol), and the mixture is stirred for 18 hours at room temperature. The reaction mixture is then worked up in the same manner as described in Example 4(C) and the resultant crude product is purified by way of column chromatography on silica gel followed by recrystallization from ethanol whereby 28 mg (10% in yield) of the title compound is obtained as pale yellow needles.

EXAMPLE 6

10-Chlorocarbonyloxy-7-ethylcamptothecin

7-Ethyl-10-hydroxycamptothecin (500 mg, 1.27 mmol) was suspended in dry dioxane (400 ml) and dissolved therein by adding triethylamine (2 ml) to the suspension under warming. This solution was stirred at room temperature while introducing thereinto phosgene prepared toties quoties by decomposing phosgene dimer (trichloromethoxychloroformate, 400 μl) in the presence of an active carbon catalyst. After 0.5 hours, consumption of the starting materials was confirmed

and insoluble matters were then removed by filtration. The solvent was distilled off under reduced pressure whereby the title compound was obtained as white powders (565 mg, 97.4%).

IR $\nu_{max}^{KBr}cm^{-1}$: 3430, 2980, 2940, 1775, 1744, 1656, 1595, 1514, 1458, 1222, 1161, 1033, 721.

EXAMPLE 7

10-Chlorocarbonyloxycamptothecin

10-Hydroxycamptothecin (700 mg, 1.92 mmol) was suspended in dry dioxane (1000 ml) and dissolved therein by adding triethylamine (2.5 ml) to the suspension under warming. The solution was stirred at room temperature while introducing thereto phosgene prepared toties quoties by decomposing phosgene dimer (trichloromethoxychloroformate, 500 μl) in the presence of an active carbon catalyst. After 0.5 hour, consumption of the starting materials was confirmed and insoluble matters were then removed by filtration. The solvent was distilled off under reduced pressure whereby the title compound was obtained as white powders (800 mg, 97.5%).

IR $\nu_{max}^{KBr}cm^{-1}$: 3450, 2970, 2930, 1775, 1740, 1665, 1590, 1502, 1222, 1186, 1045, 828.

Shown in the following Examples 8–23 is a general process for synthesizing various 10-aminocarbonyloxy-7-ethylcamptothecin compounds which comprises the steps as will be shown below.

10-Chlorocarbonyloxy-7-ethylcamptothecin (300 mg, 0.66 mmol) is suspended in dry dioxane (50 ml). To this suspension is added an amine described in each Example and the mixture is stirred under a warming or non-warming condition until the starting materials are consumed. The solvent is then removed by distillation under reduced pressure and the residue is subjected to separation and purification by the aid of column chromatography on silica gel whereby a 10-aminocarbonyloxy-7-ethylcamptothecin mentioned as a title compound of each Example is obtained.

In each Example are also given the yield of a compound obtained and characteristic physical data of the compound.

EXAMPLE 8

10-[(N-ethoxycarbonylmethylamino)carbonyloxy]-7-ethylcamptothecin

Using glycine ethyl ester (350 mg, 3.40 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (65 mg, 18.9%) was obtained.

M.P. 135°–138° C. (dec.).

^{1}H-NMR (in $CDCl_3$) δppm: 0.93 (3H, t, J=7 Hz), 1.19 (6H, t, J=7 Hz), 1.81 (2H, q, J=7 Hz), 3.00 (2H, q, J=7 Hz), 4.00–4.32 (4H, m), 5.08 (2H, s), 5.41 (2H, ABq.), 7.50 (1H, s), 7.39–8.10 (3H, m).

EXAMPLE 9

10-(2-diethylamino) ethylaminocarbonyloxy-7-ethylcamptothecin

Using N,N-diethylethylenediamine (380 mg, 3.30 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (229 mg, 65.0%) was obtained.

M.P. 154°–157° C. (dec.).

EXAMPLE 10

10-diethylaminocarbonyloxy-7-ethylcamptothecin

Using diethylamine (150 mg, 2.05 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (210 mg, 64.8%) was obtained.

M.P. 239°–242° C. (dec.).

^{1}H-NMR (in $CDCl_3$) δppm: 1.03 (3H, t, J=7 Hz), 1.25 (3H, t, J=7 Hz), 1.39 (6H, t, J=7 Hz), 1.84 (2H, q, J=7 Hz), 3.11 (2H, q, J=7 Hz), 3.44 (4H, ps. quint.), 5.16 (2H, s), 5.42 (2H, ABq.), 7.45 (1H, dxd, J=2 Hz, 8 Hz), 7.50 (1H, s), 7.71 (1H, d, J=2 Hz), 8.06 (1H, d, J=8 Hz).

IR $\nu_{max}^{KBr}cm^{-1}$: 3480, 3040, 3010, 1763, 1735, 1674, 1615, 1428, 1285, 1246, 1205, 1172, 1000, 860.

EXAMPLE 11

7-Ethyl-10-(4-morpholino)carbonyloxycamptothecin

Using morpholine (180 mg, 2.06 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (230 mg, 69.0%) was obtained.

M.P. 245°–248° C. (dec.).

^{1}H-NMR (in $CDCl_3$) δppm: 1.03 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.90 (2H, q, J=7 Hz), 3.16 (2H, q, J=7 Hz), 3.70–3.80 (8H, m), 5.25 (2H, s), 5.51 (2H, ABq.), 7.58 (1H, dxd, J=2 Hz, 8 Hz), 7.65 (1H, s), 7.84 (1H, d, J=2 Hz), 8.23 (1H, d, J=8 Hz).

IR $\nu_{max}^{KBr}cm^{-1}$: 3440, 2970, 1715, 1655, 1603, 1412, 1226, 1185, 1160, 1116, 1054, 940.

EXAMPLE 12

7-Ethyl-10-(1-piperazino)carbonyloxycamptothecin

Using piperazine (300 mg, 3.48 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (85 mg, 25.5%) was obtained.

M.P. 228°–230° C. (dec.).

^{1}H-NMR (in DMSO-d_6) δppm: 0.90 (3H, t, J=7 Hz, 1.32 (3H, t, J=7 Hz), 1.97 (2H, q, J=7 Hz), 3.04–3.65 (10H, m), 5.32 (2H, s), 5.44 (2H, s), 6.50 (1H, s), 7.34 (1H, s), 7.66 (1H, dxd, J=2 Hz, 8 Hz), 7.97 (1H, d, J=2 Hz), 8.16 (1H, d, J=8 Hz).

IR $\nu_{max}^{KBr}cm^{-1}$: 3430, 2960, 2940, 1745, 1718, 1660, 1590, 1413, 1230, 1190, 1053, 840.

Elementary analysis as $C_{27}H_{28}N_4O_6.H_2O$: Calcd. C, 62.05; H, 5.79; N, 10.72; Found C, 62.02; H, 5.42; N, 10.96.

EXAMPLE 13

7-Ethyl-10-(4-methyl-1-piperazino)carbonyloxycamptothecin

Using N-methylpiperazine (200 mg, 2.02 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (185 mg, 54.2%) was obtained.

M.P. 236°–239° C. (dec.).

^{1}H-NMR (in DMSO-d_6) δppm: 0.88 (3H, t, J=7 Hz), 1.29 (3H, t, J=7 Hz), 1.87 (2H, q, J=7 Hz), 2.25 (3H, s), 3.18 (2H, q, J=7 Hz), 3.49–3.64 (8H, m), 5.31 (2H, s), 5.43 (2H, s), 6.50 (1H, s), 7.31 (1H, s), 7.64 (1H, dxd, J=2 Hz, 9 Hz), 7.97 (1H, d, J=2 Hz), 8.15 (1H, d, J=9 Hz).

IR $\nu_{max}^{KBr}cm^{-1}$: 3430, 2970, 2940, 1743, 1715, 1655, 1598, 1459, 1412, 1292, 1228, 1190, 1052, 1001, 841, 817.

Elementary analysis as $C_{30}H_{34}N_4O_7.\frac{1}{2}H_2O$: Calcd. C, 63.75; H, 5.92; N, 10.62 Found C, 63.87; H, 5.74; N, 10.71.

EXAMPLE 14

7-Ethyl-10-(4-ethyl-1-piperazino)carbonyloxycamptothecin

Using N-ethylpiperazine (230 mg, 2.03 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (264 mg, 75.3%) was obtained.

M.P. 200°-203° C. (dec.).

^{1}H-NMR (in DMSO-d_6) δppm: 0.90 (3H, t, J=7 Hz), 1.06 (3H, t, J=7 Hz), 1.32 (3H, t, J=7 Hz), 1.90 (2H, q, J=7 Hz), 2.42 (2H, q, J=7 Hz), 3.18-3.17 (10H, m), 5.33 (2H, s), 5.44 (2H, s), 6.48 (1H, s), 7.35 (1H, s), 7.66 (1H, dxd, J=2 Hz, 8 Hz), 7.99 (1H, d, J=2 Hz), 8.18 (1H, d, J=8 Hz).

IR $\nu_{max}^{KBr}cm^{-1}$: 3430, 2960, 2930, 1742, 1720, 1655, 1597, 1412, 1206, 1185, 1162, 817.

EXAMPLE 15

10-(4-Benzyl-1-piperazino)carbonyloxy-7-ethylcamptothecin

Using N-benzylpiperazine (290 mg, 1.65 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (320 mg, 81.8%) was obtained.

M.P. 160°-162° C. (dec.).

^{1}H-NMR (in DMSO-d_6) δppm: 0.89 (3H, t, J=7 Hz), 1.29 (3H, t, J=7 Hz), 1.87 (2H, q, J=7 Hz), 3.19 (2H, q, J=7 Hz), 3.56 (2H, s), 3.50-3.70 (8H, m), 5.32 (2H, s), 5.43 (2H, s), 6.50 (1H, s), 7.32 (1H, s), 7.34 (5H, s), 7.45 (1H, dxd, J=8 Hz, 2 Hz), 7.97 (1H, d, J=2 Hz), 8.16 (1H, d, J=8 Hz).

IR $\nu_{max}^{KBr}cm^{-1}$: 3440, 2940, 1720, 1655, 1600, 1415, 1226, 1183, 1055, 1000.

Elementary analysis as $C_{34}H_{34}N_4C_6.H_2O$: Calcd. C, 66.65; H, 5.92; N, 9.14; Found C, 67.13; H, 5.62; N, 9.37.

EXAMPLE 16

7-Ethyl-10-[4-(4-methoxyphenyl)-1-piperazino]carbonyloxycamptothecin

Using N-(4-methoxyphenyl)piperazine (380 mg, 1.98 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (255 mg, 63.3%) was

M.P. 156°-158° C. (dec.).

^{1}H-NMR (in DMSO-d_6) δppm: 0.89 (3H, t, J=7 Hz), 1.30 (3H, t, J=7 Hz), 1.88 (2H, q, J=7 Hz), 3.14 (6H, br.s), 3.71 (3H, s), 3.72 (4H, br.s), 5.32 (2H, s), 5.44 (2H, s), 6.50 (1H, s), 6.91 (4H, ABq), 7.32 (1H, s), 7.69 (1H, dxd, J=2 Hz, 8 Hz), 8.01 (1H, d, J=2 Hz), 8.18 (1H, d, J=8 Hz).

IR $\nu_{max}^{KBr}cm^{-1}$: 3440, 2970, 2940, 1745, 1720, 1658, 1600, 1515, 1415, 1228, 1196, 1160, 1035, 825.

EXAMPLE 17

7-Ethyl-10-[4-(3-hydroxypropyl)-1-piperazino]carbonyloxycamptothecin

Using N-(3-hydroxypropyl)piperazine (300 mg, 2.08 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (180 mg, 48.5%) was obtained.

M.P. 228°-230° C. (dec.).

^{1}H-NMR (in DMSO-d_6) δppm: 0.89 (3H, t, J=3 Hz), 1.30 (3H, t, J=7 Hz), 1.63 (2H, m), 1.88 (2H, q, 7 Hz), 3.20-3.65 (14H, m), 5.32 (2H, s), 5.43 (2H, s), 6.51 (1H, s), 7.32 (1H, s), 7.65 (1H, dxd, J=2 Hz, 8 Hz), 7.98 (1H, d, J=2 Hz), 8.17 (1H, d, J=8 Hz).

IR $\nu_{max}^{KBr}cm^{-1}$: 3300, 2940, 1709, 1655, 1592, 1412, 1228, 1185, 1055, 815.

EXAMPLE 18

7-Ethyl-10-[4-(isopropylcarbamoylmethyl)-1-piperazino]carbonyloxycamptothecin

Using N-(isopropylcarbamoylmethyl)piperazine (370 mg, 2.00 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (133 mg, 33.4%) was obtained.

M.P. 237-240° C. (dec.).

^{1}H-NMR (in DMSO-d_6) δppm: 0.89 (3H, t, J=7 Hz), 1.09 (6H, d, J=6 Hz), 1.30 (3H, t, J=7 Hz), 1.88 (2H, q, J=7 Hz), 2.60 (4H, br.s), 3.23 (2H, s), 3.40-3.70 (4H, m), 3.70-4.00 (1H, m), 5.32 (2H, s), 5.43 (2H, s), 6.50 (1H, s), 7.32 (1H, s), 7.56 (1H, d, J=8 Hz), 7.65 (1H, dxd, J=2 Hz, 8 Hz), 7.98 (1H, d, J=2 Hz), 8.16 (1H, d, J=8 Hz).

IR $\nu_{max}^{KBr}cm^{-1}$: 3420, 3340, 2960, 1750, 1720, 1655, 1595, 1225, 1182, 1052.

Elementary analysis as $C_{32}H_{37}N_5O_7.H_2O$: Calcd. C, 61.88; H, 6.33; N, 11.28, Found C, 61.89; H, 6.33; N, 11.28.

EXAMPLE 19

7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin

Using 4-piperidinopiperidine (330 mg, 1.96 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (154 mg, 39.8%) was obtained.

M.P. 215°-218° C. (dec.).

^{1}H-NMR (in $CDCl_3$) δppm: 1.03 (3H, t, J=7 Hz), 1.40 (3H, t, J=7 Hz), 1.50-2.20 (16H, m), 2.50-2.60 (4H, m), 3.16 (2H, q, J=7 Hz), 4.38 (1H, br.s), 5.25 (2H, s), 5.52 (2H, ABq), 7.58 (1H, dxd, J=2 Hz, 9 Hz), 7.64 (1H, s), 7.83 (1H, d, J=2 Hz), 8.21 (1H, d, J=9 Hz).

IR $\nu_{max}^{KBr}cm^{-1}$: 3420, 2930, 1715, 1655, 1600, 1412, 1224, 1180, 1160, 1020, 800.

Elementary analysis as $C_{33}H_{38}N_4O_6.H_2O$: Calcd. C, 65.54; H, 6.67; N, 9.27; Found C, 65.28; H, 6.39; N, 9.39.

EXAMPLE 20

7-Ethyl-10-[N-methyl-N-(2-dimethylaminoethyl)-]aminocarbonyloxycamptothecin

Using N,N,N'-trimethylethylenediamine (200 mg, 1.96 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (168 mg, 48.9%) was obtained.

^{1}H-NMR (in $CDCl_3$) δppm: 1.03 (3H, t, J=7 Hz), 1.39 (3H, t, J=7 Hz), 1.84 (2H, q, J=7 Hz), 2.36 (6H, br.s), 2.64 (2H, q, J=6 Hz), 3.09, 3.22 (3H, s, s), 3.16 (2H, q, J=6 Hz), 3.58 (2H, t, J=7 Hz), 5.24 (2H, s), 5.27, 5.75 (2H, d, d, J=16 Hz), 7.26 (1H, s), 7.41 (1H, d, d, J=2 Hz, J=9 Hz), 7.62 (1H, d, J=2 Hz), 8.09 (1H, d, J=9 Hz).

EXAMPLE 21

7-Ethyl-10-[N-methyl-N-(1-methyl-4-piperidino)amino]carbonyloxycamptothecin

Using methyl-1-methyl-4-piperidylamine (250 mg, 1.95 mmol) as the amine, the reaction followed by the after-treatment was carried out whereby the title compound (221 mg, 60.8%) was obtained.

M.P. 159°–162° C. (dec.).

^{1}H-NMR (in $CDCl_3$) δppm: 1.03 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.80–2.15 (6H, m), 2.04 (3H, s), 3.06 (3H, s), 3.00–3.20 (6H, m), 4.12 (1H, q, J=7 Hz), 5.25 (2H, s), 5.52 (2H, ABq), 7.59 (1H, dxd, J=2 Hz, 8 Hz), 7.65 (1H, s), 7.85 (1H, d, J=2 Hz), 8.22 (1H, d, J=8 Hz).

IR ν_{max}^{KBr}cm^{-1}: 3420, 2940, 2800, 1745, 1720, 1656, 1600, 1405, 1365, 1322, 1232, 1188, 1160, 1112, 992, 822.

EXAMPLE 22

10-(4-Morpholino)carbonyloxycamptothecin

10-Chlorocarbonyloxycamptothecin (200 mg, 0.469 mol) was suspended in dry dioxane (50 ml). To this suspension was added morpholine (180 mg, 2.06 mmol), and the mixture was stirred for 3 hours at room temperature. The solvent was then removed by distillation under reduced pressure and the residue was subjected to column chromatography on silica gel for separation and purification whereby the title compound (111 mg, 49.9%) was obtained.

M.P. 277°–279° C.

^{1}H-NMR (in $CDCl_3$) δppm: 1.01 (3H, t, J=7 Hz), 1.87 (2H, q, J=7 Hz), 3.40–3.90 (8H, m), 5.18 (2H, s), 5.41 (2H, ABq), 7.46 (1H, dxd, J=2 Hz, 9 Hz), 7.52 (1H, s), 7.55 (1H, d, J=2 Hz), 8.07 (1H, d, J=9 Hz), 8.15 (1H, s).

IR ν_{max}^{KBr}cm^{-1}: 3400, 2960, 2920, 2850, 1750, 1718, 1653, 1598, 1415, 1360, 1222, 1190, 1146, 1118, 1055, 853, 746.

EXAMPLE 23

10-(4-Methyl-1-piperazino)carbonyloxycamptothecin

Using N-methylpiperazine (200 mg, 2.02 mmol) in place of morpholine in Example 22, the reaction followed by the after-treatment was carried out in the same manner as described in Example 22 whereby the title compound (141 mg, 61.3%) was obtained.

M.P. 279°–281° C. (dec.).

^{1}H-NMR (in $CDCl_3$) δppm: 1.02 (3H, t, J=7 Hz), 1.87 (2H, q, J=7 Hz), 2.32 (3H, s), 2.40 (4H, t, J=5 Hz), 3.50–3.90 (4H, m), 5.18 (2H, s), 5.41 (2H, ABq), 7.45 (1H, dxd, J=2 Hz, 8 Hz), 7.54 (1H, d, J=2 Hz), 7.66 (1H, s), 8.06 (1H, d, J=8 Hz), 8.14 (1H, s).

IR ν_{max}^{KBr}cm^{-1}: 3430, 2940, 2800, 1740, 1704, 1660, 1608, 1428, 1290, 1230, 1192, 1154, 1058, 1000, 838, 816.

EXAMPLE 24

7-Ethyl-10-(4-propyl-1-piperazino)carbonyloxycamptothecin

7-Ethyl-10-(1-piperazino)carbonyloxycamptothecin (80 mg, 0.156 mmol) was stirred in a mixed solvent of methylene chloride and ethanol with propyl bromide (200 μl) for 2 hours at room temperature in the presence of potassium carbonate (50 mg). Thereafter, insoluble matters were removed by filtration and the solvent was distilled off under reduced pressure from the filtrate. The residue was subjected to column chromatography on silica gel for purification whereby the title compound (30 mg, 34.7%) was obtained.

M.P. 210°–213° C. (dec.).

^{1}H-NMR (in $CDCl_3$) δppm: 0.93 (3H, t, J=7 Hz), 1.02 (3H, t, J=7 Hz), 1.38 (3H, t, J=7 Hz), 1.50–1.60 (2H, m), 1.87 (2H, q, J=7 Hz), 2.51 (2H, t, J=7 Hz), 3.11 (2H, q, J=7 Hz), 3.50–3.90 (8H, m), 5.16 (2H, s), 5.43 (2H, ABq), 7.45 (1H, dxd, J=2 Hz, 8 Hz), 7.50 (1H, s), 7.70 (1H, d, J=2 Hz), 8.07 (1H, d, J=8 Hz).

IR ν_{max}^{KBr}cm^{-1}: 3440, 2960, 2930, 1750, 1720, 1655, 1598, 1412, 1230, 1186, 1052, 1000, 818.

EXAMPLE 25

10-[4-(Isopropylcarbamoylmethyl)-1-piperazino]carbonyloxycamptothecin

To a solution of 10-chlorocarbonyloxycamptothecin (3.30 g, 4.5 mmol) in MeOH-$CHCl_3$ (210 ml–490 ml) containing 1.09 ml (11.1 mmol) of triethylamine was added with stirring N-isopropyl-1-piperazine acetamide (1.47 g, 7.8 mmol) in portions, and the mixture was stirred for 20 hours at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residual material was passed through a silica gel column with 2%-MeOH-$CHCl_3$ as an eluent. The title compound was obtained in a yield of 0.31 g (11% yield) which was recrystallized from ethanol to give pale yellow needles.

M P. 204°–205° C. (dec.) [EtOH].

^{1}H-NMR (100 MHz, $CDCl_3$): 1.04 (t, 3H, J=6 Hz), 1.21 (d, 6H, J=6 Hz), 1.90 (q, 2H, J=6 Hz), 2.54–2.80 (m, 4H), 2.97–3.17 (s, 2H), 3.53–3.96 (m, 4H), 4.02–4.17 (m, 1H), 5.30 (s, 2H), 5.30 (d, 1H, J=15 Hz), 5.75 (d, 1H, J=15 Hz), 6.68–7.03 (m, 1H), 7.56 (q, 1H, J=3 Hz, 9 Hz), 7.64 (s, 1H), 7.66 (d, 1H, J=3 Hz), 8.19 (d, 1H, J=9 Hz), 8.25 (s, 1H).

MS m/e: 531 [M^+-CO_2].

Elementary analysis as $C_{30}H_{33}N_5O_7$: Calcd. C, 62.60; H, 5.78; N, 12.17; Found C, 62.47; H, 5.56; N, 12.00.

EXAMPLE 26

10-[4-(1-Piperidino)piperidino]carbonyloxycamptothecin

To a solution of 10-hydroxycamptothecin (364 mg, 1 mmol) in dry pyridine (25 ml) was added 1-chlorocarbonyl-4-piperidinopiperidine (395 mg, 1.97 mmol), and the mixture was stirred for 1 hour at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residue was dissolved in $CHCl_3$ (200 ml). The solution was washed successively with a 7% aqueous solution of $NaHCO_3$ (100 ml), a saturated aqueous solution of NaCl (100 ml) and the $CHCl_3$ layer was dried with anhydrous $MgSO_4$, filtered, and evaporated in vacuo. The residual material was decolorized by passing it through a short silica gel column whereby 420 mg (75% in yield) of the title compound was obtained.

M.P. 201° C. (dec.).

MS m/z: 514, 195.

^{1}H-NMR (100 MHz, $CDCl_3$) δppm: 1.03 (t, 3H, J=7 Hz), 1.29–1.88 (m, 10H), 1.89 (q, 2H, J=7 Hz), 2.61 (br., 5H), 2.83–3.22 (m, 2H), 3.88–4.09 (s, 1H), 4.20–4.59 (m, 2H), 5.25 (s, 2H), 5.27 (d, 1H, J=16 Hz), 5.71 (d, 1H, J=16 Hz), 7.52 (dd, 1H, J=3 Hz,

9 Hz), 7.59 (s, 1H), 7.60(d, 1H, J=3 Hz), 8.12 (d, 1H, J=9 Hz), 8.22 (s, 1H).

IR $\nu_{max}^{KBr}cm^{-1}$: 1752, 1719, 1656, 1600, 1226, 1190, 1146.

EXAMPLE 27

7-Chloro-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin

7-Chloro-10-hydroxycamptothecin (110 mg, 0.280 mmol) and 1-chlorocarbonyl-4-piperidinopiperidine (100 mg, 0.42 mmol) were dissolved in anhydrous pyridine (12 ml) and the mixture was stirred for 1 hour at room temperature. The reaction mixture was evaporated to dryness in vacuo, and the residue was dissolved in $CHCl_3$ (100 ml). The $CHCl_3$ solution was shaken with a 7% aqueous solution of $NaHCO_3$ (100 ml), a saturated aqueous solution of NaCl, dried with $MgSO_4$, filtered and then evaporated to dryness in vacuo. The residual material was purified by way of column chromatography on silica gel with 2% MeOH-$CHCl_3$ as an eluent to give 110 mg (66.6% in yield) of the title compound, which gave 52 mg (31.1% yield) of pale yellow needles after recrystallization from ethanol.

^{1}H-NMR (in $CDCl_3$) δppm: 1.04 (3H, t, J=7 Hz), 1.55–1.70 (13H, m), 1.99 (2H, q, J=7 Hz), 3.64 (1H, m), 5.32 (2H, s), 5.55 (2H, dxd, J=15 Hz), 7.63 (1H, s), 7.66 (1H, dxd, J=2 Hz, 9 Hz), 8.04 (1H, d, J=2 Hz), 8.24 (1H, d, J=9 Hz).

MS m/e: 592[M+], 594[M+ +2].

IR $\nu_{max}^{KBr}cm^{-1}$: 3350, 2920, 1745, 1700, 1650, 1595, 1420, 1220, 1152, 1048, 842.

EXAMPLE 28

7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin

7-Ethyl-10-hydroxycamptothecin (790 mg, 2.01 mmol) and 1-chlorocarbonyl-4-piperidinopiperidine (910 mg, 3.95 mmol) were dissolved in anhydrous pyridine (50 ml), and the mixture was stirred for 1 hour at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residue was dissolved in $CHCl_3$ (200 ml). The solution was washed successively with a 7% aqueous solution of $NaHCO_3$ (200 ml), a saturated aqueous solution NaCl, and the $CHCl_3$ layer was dried with $MgSO_4$, filtered, and evaporated in vacuo. The residual material was decolorized by passing it through a short silica gel column whereby 1.11 g (94.8% in yield) of the title compound was obtained as a pale yellow mass, which was recrystallized from ethanol (ca. 60 ml) to give colorless needles (750 mg, 63.5% in yield).

EXAMPLE 29

7-Ethyl-10-[4-(isopropylcarbamoylmethyl)-1-piperazino]carbonyloxycamptothecin

7-Ethyl-10-hydroxycamptothecin (500 mg, 1.27 mmol) and 1-chlorocarbonyl-4-(isopropylcarbamoylmethyl)piperazine (633 mg, 2.56 mmol) were dissolved in anhydrous pyridine (30 ml) and the mixture was stirred for 15 hours at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residue was shaken with $CHCl_3$ (300 ml) and a 7% aqueous solution of $NaHCO_3$ (300 ml). The $CHCl_3$ layer was separated, washed with a saturated aqueous solution of NaCl (300 ml), dried with $MgSO_4$, filtered, and then evaporated to dryness in vacuo. The residual material was purified by way of column chromatography on silica gel with 2% MeOH-$CHCl_3$ as an eluent whereby a pale yellow mass was obtained, which was recrystallized from ethanol to give 600 mg (75.9% yield) of the title compound as pale yellow needles.

EXAMPLE 30

7-Ethyl-10-[4-(pyrrolidinocarbonylmethyl)-1-piperazino]carbonyloxycamptothecin

To a suspension of 10-chlorocarbonyloxy-7-ethylcamptothecin (300 mg, 0.66 mmol) in dry dioxane (50 ml) was added 1-pyrrolidinocarbonylmethylpiperazine (430 mg, 2.2 mmol), and the mixture was stirred for 12 hours at room temperature. The reaction mixture was evaporated to dryness in vacuo and residual material was purified by way of column chromatography on silica gel with 2% MeOH-$CHCl_3$ as an eluent whereby 180 mg (45% in yield) of the title compound was obtained.

M.P. 165.5°–166.5° C. (dec.) [EtOH].

^{1}H-NMR (in $CDCl_3$) δppm: 1.03 (3H, t, J=8 Hz), 1.43 (3H, t, J=8 Hz), 1.75 (2H, q, J=8 Hz), 1.93 (4H, m), 2.73 (4H, m), 3.20 (2H, q, J=8 Hz), 3.26 (2H, s), 3.53 (4H, t, J=6 Hz), 3.80 (4H, m), 5.30 (2H, s), 5.33, 5.80 (1H, d,d, J=16.5 Hz), 7.58 (1H, d, J=3 Hz), 7.70 (2H, m), 7.90 (1H, d, J=3 Hz), 8.28 (1H, d, J=3 Hz).

Elementary analysis as $C_{33}H_{37}N_5O_7.H_2O$: Calcd. C, 62.55; H, 6.20; N, 11.05; Found C, 62.45; H, 6.05; N, 11.12.

EXAMPLE 31

7-Ethyl-10-[4-(morpholinocarbonylmethyl)-1-piperazinocarbonyloxycamptothecin

To a suspension of 10-chlorocarbonyloxy-7-ethylcamptothecin (300 mg, 0.66 mmol) in dry dioxane (50 ml) was added 1-morpholinocarbonylmethylpiperazine (470 mg, 2.2 mmol), and the mixture was stirred for 18 hours at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residual material was purified by way of column chromatography on silica gel with 2% MeOH-$CHCl_3$ as an eluent whereby 230 mg (55% in yield) of the title compound was obtained, which gave pale yellow needles by recrystallization from ethanol.

M.P. 205.5°–208° C. (dec.) [EtOH].

^{1}H-NMR (in $CDCl_3$) δppm: 1.07 (3H, t, J=8 Hz), 1.43 (3H, t, J=8 Hz), 1.86 (2H, q, J=8 Hz), 2.70 (4H, m), 3.28 (2H, q, J=8 Hz), 3.33 (2H, s), 3.70 (12H, br.s), 5.30 (2H, s), 5.35, 5.83 (2H, dxd, J=16.5 Hz), 7.63 (1H, d, J=3 Hz), 7.93 (1H, d, J=3 Hz), 8.32 (1H, d, J=9 Hz).

Elementary analysis as $C_{33}H_{37}N_5O_8.\frac{1}{2}H_2O$: Calcd. C, 61.86; H, 5.98; N, 10.93; Found C, 62.06; H, 5.78; N, 10.94.

EXAMPLE 32

7-Ethyl-10-[(2-carboxy)-1-pyrrolidino]carbonyloxycamptothecin

To a solution of 10-chlorocarbonyloxy-7-ethylcamptothecin (1.88 g, 4.12 mmol) in anhydrous pyridine (100 ml), was added L-proline (568 mg, 4.94 mmol), and the mixture was stirred for 48 hours at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residue was purified by way of column chromatography on silica gel with 2% MeOH-$CHCl_3$ as

an eluent whereby 610 mg (27.8% in yield) of the title compound was obtained as yellow crystals.

^{1}H-NMR (in DMSO-d_6) δppm: 0.88 (3H, t, J=7 Hz), 1.29 (3H, t, J=7 Hz), 1.80–2.20 (4H, m), 2.90–3.60 (6H, m), 5.24 (2H, s), 5.41 (2H, s), 6.47 (1H, s, D_2O-exchangeable), 7.23 (1H, s), 7.27–8.03 (3H, m), 8.31 (1H, s).

MS m/e: 504 [M$^+$-29], 489 [M$^+$-44].

EXAMPLE 33

10-[4-isopropylcarbamoylmethyl)-1-piperazino]carbonyloxy-7-propylcamptothecin

To a solution of 10-hydroxy-7-propylcamptothecin (390 mg, 1 mmol) in dry pyridine (50 ml) was added 1-chlorocarbonyl-4-(isopropylcarbamoylmethyl)piperazine (444 mg, 1.8 mmol), and the mixture was stirred for 16 hours at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residue was dissolved in $CHCl_3$ (250 ml). The solution was washed successively with a 7% aqueous solution of $NaHCO_3$ (350 ml) and a saturated aqueous solution of NaCl (200 ml) dried with anhydrous $MgSO_4$, filtered, and then evaporated to dryness in vacuo. The residue was purified by way of column chromatography on silica gel with 2% MeOH-$CHCl_3$ as an eluent whereby 462 mg (75% in yield) of the title compound was obtained.

M.P. 226°–229° C.

MS m/z: 573 [M$^+$-44], 487 [base, 573-$(CH_3)_2CHNHCO$].

^{1}H-NMR (100 MHz, $CDCl_3$) δppm: 1.04 (t, 3H, J=6 Hz), 1.10 (t, 3H, J=6 Hz), 1.22 (d, 6H, J=7 Hz), 1.70–2.11 (m, 4H), 2.51–2.87 (m, 4H), 2.90–3.30 (m, 4H), 3.52–3.93 (m, 4H), 3.98 (s, 1H), 3.99–4.35 (m, 1H), 5.24 (s, 2H), 5.29 (d, 1H, J=16 Hz), 5.75 (d, 1H, J=16 Hz), 6.70–7.45 (m, 1H), 7.55 (dd, 1H, J=2 Hz, 9 Hz), 7.62 (s, 1H), 7.78 (d, 1H, J=2 Hz), 8.18 (d, 1H, J=9 Hz).

IR ν_{max}^{KBr}cm^{-1}: 1753, 1720, 1656, 1592, 1227, 1205, 1178, 1155.

EXAMPLE 34

7-Butyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin

7-Butyl-10-hydroxycamptothecin (386 mg, 0.9 mmol) and 4-piperidinopiperidino-1-carbonyl chloride (320 mg, 1.4 mmol) were dissolved in anhydrous pyridine (ca. 20 ml), and the mixture was stirred for 45 min. at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residual material was purified by way of column chromatography on silica gel with 2% MeOH-$CHCl_3$ as an eluent whereby 360 mg (66.6% in yield) of the title compound was obtained which was recrystallized from ethanol to give pale yellow needles (137 mg, 24% in yield).

M.P. 204–207 (dec.) [EtOH].

^{1}H-NMR (100 MHz, $CDCl_3$) δppm: 0.90–1.22 (m, 6H), 1.32–2.32 (m, 16H), 2.51–2.90 (m, 5H), 2.91–3.38 (m, 4H), 4.04 (s, 1H), 4.24–4.73 (m, 2H), 5.25 (s, 2H), 5.30 (d, 1H, J=16 Hz), 5.76 (d, 1H, J=16 Hz), 7.56 (dd, 1H, J=3 Hz, 9 Hz), 7.65 (s, 1H), 7.78 (d, 1H, J=3 Hz), 8.18 (d, 1H, J=9 Hz).

IR ν_{max}^{KBr}cm^{-1}: 1754, 1719, 1653, 1596, 1224, 1196, 1180, 1153.

MS m/e: 614 [M$^+$], 570 [M$^+$—CO_2].

Elementary analysis as $C_{35}H_{42}O_6N_2$: Calcd. C, 68.38; H, 6.89; N, 9.12; Found C, 68.09; H, 6.87; N, 8.83.

EXAMPLE 35

7-Ethyl-10-(4-methyl-1-piperazino)carbonyloxycamptothecin hydrochloride

7-Ethyl-10-(4-methyl-1-piperazino)carbonyloxycamptothecin (500 mg, 0.97 mmol) obtained in Example 13 was dissolved in ethanol (10 ml) containing 9 ml of 0.1N HCl, and the solution was passed through a filter (0.5 μm in pore size, Millex-SR). The filtrate was evaporated to dryness in vacuo at 40° C. and the residual yellow powder was recrystallized from absolute ethanol whereby 373 mg (74% in yield) of the title compound was obtained as yellow needles. The salt was freely soluble in water and an aqueous solution of the salt (25 mg/ml) showed a pH value of about 6.

EXAMPLE 36

7-Ethyl-10-(1-piperazino)carbonyloxycamptothecin sulfate

To an ice-cooled suspension in distilled water (18 ml) of 7-ethyl-10-(1-piperazino)carbonyloxycamptothecin (1.00 g, 1.98 mmol) obtained in Example 12 was added 1/10N H_2SO_4 (17.9 ml, 0.895 mmol), and the suspended solution was stirred vigorously for 5 minutes under cooling in an ice bath. The solution was passed through a filter (0.22 μm, SLGS 025 OS) and the filtrate was lyophilized overnight (−40°–+25° C., 10 mmHg) whereby 985 mg (96% in yield) of the title compound was obtained as a pale yellow amorphous solid.

EXAMPLE 37

7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin hydrochloride

To an ice-cooled suspension in distilled water (15 ml) of 7-ethyl-10-[1-(4-piperidino)piperidino]carbonyloxycamptothecin (1.00 g, 1.7 mmol) obtained in Example 19 was added 1/10N HCl (15.3 ml, 1.53 mmol), and the suspension was stirred vigorously for 5 minutes under cooling in an ice bath. The solution was passed through a filter (0.22 μm, SLGS 025 OS) and the filtrate was lyophilized overnight (−40°–25° C., 10 mmHg) whereby 950 mg (89.8% in yield) of the title compound was obtained as a pale yellow amorphous solid.

IR ν_{max}^{KBr}cm^{-1}: 3400, 2950, 2650, 1740(sh), 1710, 1650, 1595, 1410, 1220, 1180, 1040.

EXAMPLE 38

7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin sulfate

To an ice-cooled suspension in distilled water (15 ml) of 7-ethyl-10-[1-(4-piperidino)piperidino]carbonyloxycamptothecin (1.00 g, 1.7 mmol) obtained in Example 19 was added 1/10N H_2SO_4 aq (15.3 ml, 7.65 mmol), and the suspended solution was stirred vigorously for 5 minutes under cooling in an ice bath. The solution was passed through a filter (0.22 μm, SLGS 025 OS) and the filtrate was lyophilized overnight (−40°–+25° C., 10 mmHg) whereby 970 mg (90% in yield) of a pale yellow amorphous solid which was recrystallized from absolute ethanol to give pale yellow needles (776 mg, 71.8% in yield).

M.P. 205°–207° C. (dec.) [EtOH].

IR ν_{max}^{KBr}cm^{-1}: 3400, 2920, 1740(sh), 1750, 1650, 1595, 1410, 1215, 1180, 1150, 1100, 1010.

Elementary analysis as $C_{33}H_{38}N_4O_6 \cdot \frac{1}{2}H_2SO_4 \cdot 5H_2O$: Calcd. C, 58.22; H, 6.52; N, 8.23; Found C, 58.11; H, 6.24; N, 8.23.

EXAMPLE 39

7-Ethyl-10-[4-(isopropylcarbamoylmethyl)-1-piperazino]carbonyloxycamptothecin sulfate

To an ice-cooled suspension in distilled water (14 ml) of 7-ethyl-10-[4-(isopropylcarbamoylmethyl)-1-piperazino]carbonyloxycamptothecin (1.00 g, 1.6 mmol) obtained in Example 18 was added 1/10 N H_2SO_4 (14.5 ml, 7.25 mmol), and the suspension was stirred vigorously for 5 minutes under cooling in an ice bath. The solution was passed through a filter (0.22 μm, SLGS 025 OS) and the filtrate was lyophilized overnight (−40°−+25° C., 10 mmHg) whereby 965 mg (96% in yield) of the title compound was obtained as a pale yellow amorphous solid.

EXAMPLE 40

7-Ethyl-10-[4-(isopropylcarbamoylmethyl)-1-piperazino]carbonyloxycamptothecin methanesulfonate

To a solution of 7-ethyl-10-[4-(isopropylcarbamoylmethyl)-1-piperazino]carbonyloxycamptothecin (200 mg, 0.32 mmol) obtained in Example 18 in ethanol (100 ml) was added 1/10N methanesulfonic acid (3 ml, 0.3 mmol), and the resulting yellow solution was passed through a filter (0.5 μm, Millex-SR). The filtrate was evaporated to dryness in vacuo at about 40° C. The residual material was recrystallized from ethanol to give 520 mg (72% in yield) of the title compound as yellow prisms.

IR $\nu_{max}^{KBr}cm^{-1}$: 3450, 2950, 1720, 1650, 1600, 1420, 1195, 1050, 960.

EXAMPLE 41

7-Ethyl-10-[(2-carboxy)-1-pyrrolidino]carbonyloxycamptothecin sodium salt

To an ice-cooled suspension in distilled water (15 ml) of 7-ethyl-10-[(2-carboxy)-1-pyrrolidino]carbonyloxycamptothecin (1.00 g, 1.88 mmol) obtained in Example 32 was added 1/10N NaOH (16.9 ml, 1.69 mmol), and the suspension was stirred vigorously for 5 minutes under cooling in ice-bath. The solution was allowed to pass through a filter (0.22 μm, SLGS 0.25 OS) and the filtrate was lyophilized overnight (−40° C.−+25° C., 10 mmHg) whereby 872 mg (85.5% in yield) of the title compound was obtained as a pale yellow amorphous solid.

M.P. 232°−234° C. (dec.) [EtOH].

EXAMPLE 42

11-[4-(1-Piperidino)-1-piperidino]carbonyloxycamptothecin

11-Hydroxycamptothecin (35 mg, 0.096 mmol) and 1-chlorocarbonyl-4-piperidinopiperidine (45 mg, 0.195 mmol) were dissolved in anhydrous pyridine (2 ml), and the mixture was stirred for 45 minutes at room temperature. After removal of the solvent by evaporation, the residual material was purified by way of preparative thin layer chromatography using 10% MeOH-$CHCl_3$ as a solvent whereby 27 mg (50.4% in yield) of the title compound was obtained.

^{1}H-NMR (in $CDCl_3$) δppm: 1.03 (3H, t, J=7 Hz), 1.60–2.50 (18H, m), 4.43 (1H, br.s), 5.29 (2H, s), 5.54 (2H, d,d, J=15 Hz), 7.52 (1H, d,d, 2 Hz, 8 Hz), 7.65 (1H, s), 7.84–7.95 (2H, m), 8.38 (1H, s).

MS m/e: 558 [M$^+$].

EXAMPLE 43

11-[4-(Isopropylcarbamoylmethyl)-1-piperazino]carbonyloxycamptothecin

11-Hydroxycamptothecin (35 mg, 0.096 mmol) and 1-chlorocarbonyl-4-(isopropylcarbamoylmethyl)piperazine (50 mg, 0.202 mmol) were dissolved in dry pyridine (2 ml), and the mixture was stirred for 16 hours at room temperature. After removal of the solvent by evaporation, the residual material was purified by way of preparative thin layer chromatography using 10% MeOH-$CHCl_3$ as a solvent whereby 37 mg (68% in yield) of the title compound was obtained.

EXAMPLE 44

7-Ethyl-11-[1-(4-piperidino)piperidino]carbonyloxycamptothecin

7-Ethyl-11-hydroxycamptothecin (45 mg, 0.114 mmol) and 1-chlorocarbonyl-4-piperidinopiperidine (53 mg, 0.228 mmol) were dissolved in dry pyridine (3 ml), and the mixture was stirred for 1 hour at room temperature. After removal of the solvent by evaporation, the residual material was purified by way of preparative thin layer chromatography using 10% MeOH-$CHCl_3$ as a solvent whereby 36 mg (53.8% in yield) of the title compound was obtained.

^{1}H-NMR (in $CDCl_3$) δppm: 1.30 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.55–2.55 (20H, m), 3.15 (2H, q, J=7 Hz), 4.40 (1H, br.s), 5.26 (2H, s), 5.53 (2H, dxd, 16 Hz), 7.50 (1H, m), 7.65 (1H, s), 7.92 (1H, d, J=2 Hz), 8.12 (1H, d, J=9 Hz).

EXAMPLE 45

11-(4-Ethyl-1-piperazino)carbonyloxycamptothecin

11-Hydroxycamptothecin (82 mg, 0.225 mmol) and 1-chlorocarbonyl-4-ethylpiperazine (65 mg, 0.369 mmol) were dissolved in dry pyridine (5 ml), and the mixture was stirred for 2 hours at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residual material was dissolved in $CHCl_3$ (25 ml). The $CHCl_3$ solution was washed successively with a 7% aqueous solution of $NaHCO_3$ (50 ml), a saturated aqueous solution of NaCl, dried with anhydrous $MgSO_4$, filtered, and then evaporated to dryness in vacuo. The residue was purified by way of column chromatography on silica gel with 2% MeOH-$CHCl_3$ as an eluent whereby 73 mg (64% in yield) of the title compound was obtained as a pale yellow solid.

^{1}H-NMR (in $CDCl_3$) δppm: 1.04 (3H, t, J=7 Hz), 1.21 (3H, t, J=7 Hz), 1.98 (2H, q, J=7 Hz), 2.40 (2H, q, J=7 Hz), 3.25–3.45 (8H, m), 5.28 (2H, s), 5.52 (2H, dxd, J=15 Hz), 7.50 (1H, dxd, J=2 Hz, 9 Hz), 7.56 (1H, s), 7.85–7.95 (2H, m), 8.35 (1H, s).

MS m/e: 504 [M$^+$].

IR $\nu_{max}^{KBr}cm^{-1}$: 3380, 2920, 1715, 1652, 1600, 1403, 1210 1192, 1150, 1040, 900.

REFERENTIAL EXAMPLE 1

7-Chloro-10-hydroxycamptothecin

(A) Camptothecin 1-oxide (115 mg, 0.315 mmol) was dissolved in dry dimethylformamide (35 ml). Phosphoryl chloride (253 mg, 1.64 mmol) was added dropwise to the solution with stirring. After continuously

stirring for 1.5 hours at room temperature, the reaction mixture was evaporated to dryness in vacuo and the residual material was washed with MeOH (10 ml) whereby 96 mg (80% in yield) of the title compound was obtained as a pale yellow solid.

M.P. 271°–273° C. [MeOH-$CHCl_3$-n-hexane].

MS m/e: 382 [M+] 384 [M++2].

(B) 7-Chlorocamptothecin (2.00 g, 5.23 mmol) was dissolved in acetic acid (400 ml). To the solution was added an aqueous solution of 30% hydrogen peroxide (120 ml), and the mixture was heated at 80°–85° C. in a water bath for 5 hours. The reaction mixture was concentrated to a half of its original volume and the concentrated solution was poured into ice-water (5 l). The resulting precipitate was collected on a filter by suction and dried in vacuo for 6 hours at 60° C. over P_2O_5 as a drying agent whereby 670 mg (32.1% in yield) of 7-chlorocamptothecin 1-oxide was thus obtained.

7-Chlorocamptothecin 1-oxide (335 mg, 0.84 mmol) was dissolved in dioxane (1 l) containing 8.5 ml of N/10 H_2SO_4 and the mixture was irradiated with the light from a 450 W Hg high pressure lamp for 13 minutes under cooling with water. The resulting mixture was evaporated to dryness in vacuo and the residue was dissolved in 20% MeOH-$CHCl_3$ (20 ml). The solution was washed with water (500 ml) and the precipitate was collected on a filter by suction whereby 110 mg (32.8% in yield)of the title compound was obtained as pale yellow needles after recrystallizing from MeOH-n-hexane-$CHCl_3$.

^{1}H-NMR (in $CDCl_3$) δppm: 1.04 (3H, t, J=7 Hz), 1.99 (2H, q, J=7 Hz), 5.32 (2H, s), 5.31, 5.68 (2H, d, d, J=16 Hz), 7.56 (1H, dxd, J=2 Hz, 9 Hz), 7.95 (1H, d, J=2 Hz), 8.23 (1H, d, J=9 Hz).

MS m/e: 398 [M+], 400 [M++2].

REFERENTIAL EXAMPLE 2

7-Ethyl-11-hydroxycamptothecin

11-Hydroxycamptothecin (100 mg, 0.27 mmol) was suspended in water (3 ml). To the suspension, Conc. H_2SO_4 (1.2 ml) was added until the hydroxycamptothecin was dissolved in the propionaldehyde (50 mg, 0.862 mmol) and $FeSO_4.7H_2O$ (70 mg, 0.25 mmol) were added to the solution and then 30% H_2O_2 (120 μl, 0.85 mmol) was added dropwise to the mixture under ice-cooling with stirring. After addition of the hydrogen peroxide, the mixture was stirred for 2 hours at room temperature. The reaction mixture was poured into ice-water (250 ml), and the precipitate in the solution was extracted with $CHCl_3$ (200 ml×2). The $CHCl_3$ layer was washed with a saturated aqueous solution of NaCl, dried with $MgSO_4$, filtered, and then evaporated to dryness in vacuo. The residual material was recrystallized from ethanol to give 60 mg (59.3% in yield) of the title compound as pale yellow needles.

^{1}H-NMR (in $CDCl_3$-CD_3OH) δppm: 1.03 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.94 (2H, q, J=7 Hz), 3.20 (2H, q, J=7 Hz), 5.29, 5.70 (1H, 1H, dxd, J=16 Hz), 5.23 (2H, s), 7.33 (1H, dd, J=2 Hz, 9 Hz), 7.46 (1H, d, J=2 Hz), 7.60, (1H, s), 8.05 (1H, d, J=9 Hz).

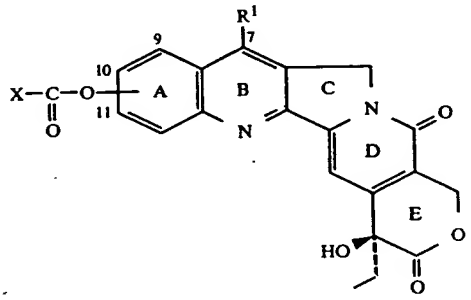
IR ν_{max}^{KBr} cm^{-1}: 3200, 2975, 2925, 1735, 1650, 1590, 1570, 1460, 1250, 1230, 1155, 1110, 1030.

It is understood that the preceeding representative examples may be varied within the scope of the present specification, both as to the reactants and conditions, by one skilled in the art to achieve essentially the same results.

As many apparently widely different embodiments of the present invention may be made without departing from the spirit and scope thereof, it is to be construed that the present invention is not limited to the specific embodiments thereof as defined in the appended claims.

What is claimed is:

1. Camptothecin derivatives of the formula:



(I)

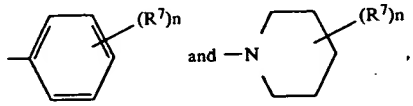
wherein R^1 is a hydrogen atom, a halogen atom or an alkyl group with 1–4 carbon atoms and X is a chlorine atom or $—NR^2R^3$ where R^2 and R^3 are the same or different and each represents a hydrogen atom, a substituted or unsubstituted alkyl group with 1–4 carbon atoms or a substituted or unsubstituted group selected from the group consisting of cyclopentyl, cyclohexyl, N-methylpiperidyl-(4), 2-pyrrolidyl, phenyl, tolyl, xylyl, pyridyl-2 and 2-methylpyridyl-(4), with the proviso that when both R^2 and R^3 are the substituted or unsubstituted alkyl groups, they may be combined together with the nitrogen atom, to which they are bonded, to form a heterocyclic ring selected from the group consisting of pyrrolidine, piperidine, 2-oxapyrrolidine, morpholine, thiomorpholine and 4-R^4 piperizine rings in which R^4 is a hydrogen atom, a substituted or unsubstitued alkyl group with 1–4 carbon atoms or a substituted or unsubstituted phenyl group and wherein the grouping —O—CO—X is bonded to a carbon atom located in any of the 9-, 10- and 11-positions in the ring A, and ammonium salts or alkali metal salts thereof.

2. The camptothecin derivatives according to claim 1, wherein R^2, R^3 or R^4 in case of the alkyl group is substituted by one or more substituents selected from the following atoms and/or groups:

—F, —Cl, —Br and —I, (A)

—OH and $—OR^5$, (B)

$—COOR^6$, $—SO_3R^6$ and $—PO_3(R^6)_2$, (C)



(D)

$—NR^8R^9$ and $—CONR^8R^9$, and (E)

(F)

$—Q—A—OR^5$, $—Q—A—NR^8R^9$ and $—Q—A—Q—R^5$

wherein R^5 is an alkyl group with 1–4 carbon atoms or a phenyl group which may be substituted by a halogen atom or an alkyl group with 1–4 carbon atoms, R^6 is a hydrogen atom or an alkyl group with 1–4 carbon atoms, R^7 is a hydrogen atom, a halogen atom, an alkyl group with 1–4 carbon atoms or an alkoxy group with 1–4 carbon atoms, n is an integer of 1–3, R^8 and R^9 are

the same or different and each represents a hydrogen atom or an alkyl group with 1–4 carbon atoms with the proviso that when both R^8 and R^9 are the alkyl groups, they may be combined together with the nitrogen atom, to which they are bonded, to from a heterocyclic ring selected from the group consisting of pyrrolidine, piperidine, 2-oxapyrrolidine, morpholine and 4-R^4-piperazine rings, Q is the grouping —O—CO— or —CO—O—, and A is a straight or branched chain alkylene group with 1–4 carbon atoms.

3. Camptothecin derivatives according to claim 1, which are 9-chlorocarbonyloxy-7-R^1-camptothecins.

4. Camptothecin derivatives according to claim 1, which are 10-chlorocarbonyloxy-7-R^1-camptothecins.

5. Camptothecin derivatives according to claim 1, which are 11-chlorocarbonyloxy-7-R^1camptothecins.

6. Camptothecin derivatives according to claim 1, which are 9-(4-R^4-1-piperazino)carbonyloxy-7-R^1-camptothecins.

7. Camptothecin derivatives according to claim 1, which are 9-(1-piperazino)carbonyloxy-7-R^1-camptothecins.

8. Camptothecin derivatives according to claim 1, which are 9-[(4-C_{1-4}alkylcarbamoylmethyl)-1-piperazino]carbonyloxy-7-R^1-camptothecins.

9. Camptothecin derivatives according to claim 1, which are 9-[4-(1-piperidino)-1-piperidino]carbonyloxy-7-R^1-camptothecins.

10. Camptothecin derivatives according to claim 1, which are 10-(di-C_{1-4}alkylamino)carbonyloxy-7-R^1-camptothecins.

11. Camptothecin derivatives according to claim 1, which are 10-[N-(di-C_{1-4}alkylamino-C_{1-4}alkyl)-]aminocarbonyloxy-7-R^1-camptothecins.

12. Camptothecin derivatives according to claim 1, which are 10-[N-C_{1-4}alkyl-N-(di-C_{1-4}alkylamino-C_{1-4}alkyl)]aminocarbonyloxy-7-R^1-camptothecins.

13. Camptothecin derivatives according to claim 1, which are 10-[N-C_{1-4}alkyl-N-(1-C_{1-4}alkyl-4-piperidino)amino]carbonyloxy-7-R^1-camptothecins.

14. Camptothecin derivatives according to claim 1, which are 10-[4-R^4-1-piperazino]carbonyloxy-7-R^1-camptothecins.

15. Camptothecin derivatives according to claim 1, which are 10-(1-piperazino)carbonyloxy-7-R^1-camptothecins.

16. Camptothecin derivatives according to claim 1, which are 10-(4-C_{1-4}alkyl-1-piperazino)carbonyloxy-7-R^1-camptothecins.

17. Camptothecin derivatives according to claim 1, which are 10-(4-phenyl-1-piperazino)carbonyloxy-7-R^1-camptothecins.

18. Camptothecin derivatives according to claim 1, which are 10-(4-benzyl-1-piperazino)carbonyloxy-7-R^1-camptothecins.

19. Camptothecin derivatives according to claim 1, which are 10-[(4-C_{1-4}alkylcarbamoylmethyl)-1-piperazino]carbonyloxy-7-R^1-camptothecins.

20. Camptothecin derivatives according to claim 1, which are 10-[4-(piperidino)-1-piperidino]carbonloxy-7-R^1-camptothecins.

21. Camptothecin derivatives according to claim 1, which are 10-(4-morpholino)carbonyloxy-7-R^1-camptothecins.

22. Camptothecin derivatives according to claim 1, which are 11-(4-R^4-1-piperazino)carbonyloxy-7-R^1-camptothecins.

23. Camptothecin derivatives according to claim 1, which are 11-(4-C_{1-4}alkyl-1-piperazino)carbonyloxy-7-R^1-camptothecins.

24. Camptothecin derivatives according to claim 1, which are 11-[(4-C_{1-4}alkylcarbamoylmethyl)-1-piperazino]carbonyloxy-7-R^1-camptothecins.

25. Camptothecin derivatives according to claim 1, which are 11-[4-(1-piperidino)-1-piperidino]carbonyloxy-7-R^1-camptothecins.

* * * * *

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PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT
4,604,463	173	490	----	06/627,980	08/05/86	07/05/84	04	NO	PAID

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1	55153P

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ROCKVILLE, MD 20850

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PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT
4,604,463	184	1870	----	06/627,980	08/05/86	07/05/84	08	NO	PAID

If the "status" column for a patent number listed above does not indicate "PAID" a code or an asterisk (*) will appear in the "status" column. Where an asterisk (*) appears, the codes are set out below by the related item number. An explanation of the codes indicated in the "status" column and as set out below by the related item number appears on the reverse of the maintenance fee statement.

ITM NBR	ATTY DKT NUMBER
1	55153P

**DIRECT THE RESPONSE TOGETHER WITH ANY QUESTIONS ABOUT THIS NOTICE TO:
COMMISSIONER OF PATENTS AND TRADEMARKS, BOX M. FEE, WASHINGTON, DC 20231**

Exhibit 4

Chronology of Events on CAMPTOSAR® (Irinotecan Hydrochloride Trihydrate) IND 35,229; NDA 20-571

DATE	EVENT
August 3, 1990	G.H. Besselar Associates submitted IND for CPT-11 on behalf of Kabushiki Kaisha Yakult Honsha.
August 6, 1990	Letter from FDA acknowledging the August 6, 1990 submission and assigning IND number 35,229.
August 15, 1990	G. H. Besselar letter submitting to FDA an additional copy of the IND.
October 22, 1990	G.H. Besselar submits Response to FDA Request for Additional Protocol Amendment: Change in Protocol New Investigator.
March 4, 1991	G.H. Besselar submits Protocol Amendment: Change in Protocol.
March 6, 1991	G.H. Besselar submits Protocol Amendment: New Protocol.
March 18, 1991	G.H. Besselar submits Other: Additional Secondary Investigators.
April 9, 1991	G.H. Besselar submits Protocol Amendment: Change in Protocol.
August 1, 1991	G.H. Besselar submits Change in Protocol (GHBA-393).
October 3, 1991	G.H. Besselar submits Protocol Amendment: New Investigator.
October 9, 1991	G.H. Besselar submits Annual Report.
October 16, 1991	G.H. Besselar submits Protocol Amendment.
January 14, 1992	G.H. Besselar submits Protocol Amendment: Change in Protocol.
October 1, 1992	G.H. Besselar submits Annual Report.

DATE	EVENT
November 4, 1992	G.H. Besselar submits Response to FDA Request for Information.
November 30, 1992	G.H. Besselar submits errors correct in the 1992 Annual Report.
December 8, 1992	Theradex submits transfer of representative agent from G.H. Besselar to Theradex.
January 11, 1993	Theradex submits Request for Compassionate Use.
January 28, 1993	Theradex submits Request for Compassionate Use.
January 28, 1993	Theradex submits Request for Compassionate Use.
February 1, 1993	Theradex submits Prorovol: Cervical Cancer Phase II, M.D. Anderson, Protocol: Colorectal Cancer.
February 26, 1993	Theradex submits Protocol Amendment: Cervical/Colorectal Cancer Phase II, Summary of IND Submission.
March 5, 1993	Theradex submits Protocol: Cervical Cancer, Loyola Univ., Labels and certificates of analysis, Revised general investigational plan.
March 9, 1993	Theradex submits Request for Compassionate Use.
March 17, 1993	Theradex submits CMC update (Type I, Osaka plant).
March 23, 1993	Theradex submits authorization for the FDA to release Theradex's name.
March 24, 1993	Theradex submits Request for Compassionate Use.
March 26, 1993	Theradex submits Request for Compassionate Use.

DATE	EVENT
April 2, 1993	Theradex submits Request for Compassionate Use.
April 8, 1993	Theradex submits Request for Compassionate Use.
April 23, 1993	Theradex submits Request for Compassionate Use.
April 29, 1993	Theradex submits Protocol: Colorectal Cancer, Mayo Clinic.
May 6, 1993	Theradex submits Request for Compassionate Use.
May 14, 1993	Theradex submits IND Safety Report on the death of pt 19 at San Antonio.
June 3, 1993	Theradex submits Request for Compassionate Use.
June 23, 1993	Theradex submits Reply to FDA Comments.
August 4, 1993	Theradex submits Request for Compassionate Use.
August 4, 1993	Theradex submits Change in Protocol (MD Andersin/Amendment 5, CTRC/Amendment 3).
August 26, 1993	Theradex submits Request for Compassionate Use.
September 21, 1993	Theradex submits Request for Compassionate Use.
October 29, 1993	Theradex submits Annual Report.
November 9, 1993	Theradex submits Change in Protocol (Mayo Clinic/Amendment 3)
November 18, 1993	Amendment No. 040, The Upjohn Company submits transfer of sponsorship along with all rights and obligations to the application from Yakult Honsha Co., Ltd., to The Upjohn Company, Kalamazoo, MI.
November 30, 1993	Amendment No. 043 - Protocol Amendment.

DATE	EVENT
December 14, 1993	Letter from Division of Oncology and Pulmonary Drug Products, FDA, transfer of IND 35,229 accepted.
January 6, 1994	Amendment No. 044 - Protocol Amendment - Compassionate Use Protocol.
February 18, 1994	Amendment No. 045 - Protocol Amendment - Special Exception Protocol.
March 2, 1994	Amendment No. 046 - Protocol Amendment.
March 25, 1994	Amendment No. 047 - New Protocol M/6475/0006.
March 31, 1994	Amendment No. 048 - New Investigator for Protocol M/6475/0006.
April 11, 1994	Amendment No. 049 - TUC requests meeting with FDA to discuss development plans
April 21, 1994	Amendment No. 050 - Teleconference (4/5/94) follow-up.
April 26, 1994	Amendment No. 051 - Special Exception Protocol CPT-11/39424.
May 3, 1994	Amendment No. 052 - Protocol Amendment.
May 18, 1994	Amendment No. 053 - Protocol Amendment.
May 20, 1994	Amendment No. 054 - Special Exception Protocol.
June 2, 1994	Amendment No. 055 - Protocol Amendment
June 2, 1994	Amendment No. 056 - Request meeting to discuss the potential for filing a NDA for the use of irinotecan to treat patients with colorectal carcinoma.
June 7, 1994	Amendment No. 057 - Protocol Amendment.
June 9, 1994	Amendment No. 058 - 10 day written report.
June 23, 1994	Amendment No. 059 - Special Exception Protocol.

DATE	EVENT
June 23, 1994	Amendment No. 060 - Clinical Information - Follow-up to Amendment No. 058, 10 day written report.
July 5, 1994	Amendment No. 061 - The Upjohn Company added as a manufacturing site for the drug product.
July 14, 1994	Amendment No. 062 - Protocol Amendment.
July 21, 1994	Amendment No. 063 - Change in Protocol.
July 22, 1994	Amendment No. 064 - Requests telephone to discuss proposed toxicity program.
July 26, 1994	Amendment No. 065 - Additional documents for the August 3 meeting with FDA.
August 1, 1994	Amendment No. 066 - Item 7. Chemistry, B. Drug Product.
August 3, 1994	Meeting with Division of Oncology and Pulmonary Drug Products to review clinical development plan leading to NDA submission. Minutes of the meeting submitted to IND in Amendment No. 071, October 7, 1994.
August 11, 1994	Amendment No. 067 - 10 day Safety Report.
August 18, 1994	Amendment No. 068 - Information Amendment.
August 22, 1994	Amendment No. 069 - Information Amendment.
September 12, 1994	Amendment No. 070 - Protocol Amendment.
October 7, 1994	Amendment No. 071 - Information Amendment and minutes of 8/3/94 FDA meeting.
October 10, 1994	Amendment No. 072 - Annual Report No. 4.
October 18, 1994	Amendment No. 073 - Protocol Amendment.
October 26, 1994	Amendment No. 074 - Protocol Amendment.

DATE	EVENT
November 11, 1994	Amendment No. 075 - Information Amendment.
November 17, 1994	Amendment No. 076 - Protocol Amendment.
November 28, 1994	Amendment No. 077 - 10 day Written Safety Report.
December 1, 1994	Amendment No. 078 - Information Amendment.
December 2, 1994	Amendment No. 079 - Proposed trademark submitted: CAMPTOSAR® Sterile Solution (irinotecan hydrochloride sterile solution).
December 5, 1994	Amendment No. 080 - Information Amendment.
December 19, 1994	Amendment No. 081 - Submission of Clinical Benefit Report in response to commitment made at August 3, 1994 FDA meeting.
December 22, 1994	Amendment No. 082 - Protocol Amendment.
January 9, 1995	Amendment No. 083 - Protocol Amendment.
January 11, 1995	Letter to James Hamilton, Office of Compliance, FDA, requesting approval to defective clinical supplies.
January 27, 1995	Amendment No. 084 - Protocol Amendment.
January 30, 1995	Facsimile sent from Upjohn to Medical Reviewer with proposal for provision of case report forms (CRFs) for foreign studies to be included in the NDA. Several interchanges between sponsor and FDA.
February 13, 1995	Telephone call from CSO relaying the following resolution on foreign CRFs to be included in the NDA: "The sponsor does not need to include CRFs from foreign phase I studies in the NDA. However, the sponsor should be prepared to submit CRFs for patients who died and

DATE	EVENT
	experienced serious adverse medical events in phase II colorectal studies conducted in France and Japan."
February 16, 1995	Amendment No. 085 - Protocol Amendment.
February 20, 1995	Amendment No. 086 - Protocol Amendment.
February 22, 1995	Amendment No. 087 - Information Amendment, Additional Data for 3/10/95 FDA meeting.
February 28, 1995	Amendment No. 088 - Protocol Amendment.
March 6, 1995	Amendment No. 089 - Information Amendment: Aseptic Processing.
March 10, 1995	Meeting with Division of Oncology and Pulmonary Drug Products to review clinical benefit data; review of U.S. clinical trial experience (tumor responses, toxicity, and management of late diarrhea); filing proposal for treatment of previously treated colorectal cancer. Minutes of the meeting submitted to IND in Amendment No. 093, April 14, 1995.
March 14, 1995	Amendment No. 090 - Protocol Amendment.
March 14, 1995	Amendment No. 091 - Protocol Amendment.
April 4, 1995	Amendment No. 092 - Protocol Amendment.
April 14, 1995	Amendment No. 093 - Protocol Amendment.
April 26, 1995	Amendment No. 094 - Protocol Amendment.
May 16, 1995	Amendment No. 095 - General correspondence.
May 18, 1995	Amendment No. 096 - Protocol Amendment.
May 22, 1995	Amendment No. 097 - IND Safety Report - 10-day Written Report.

DATE	EVENT
May 25, 1995	Amendment No. 098 - General correspondence: Request for pre-NDA meeting.
June 6, 1995	Amendment No. 099 - General correspondence: Additional copies of Pre-NDA Meeting Request Package.
June 12, 1995	Amendment No. 100 - General Correspondence: Draft Protocol for Review.
June 16, 1995	Amendment No. 101 - Protocol Amendment.
June 19, 1995	Amendment No. 102 - Information Amendment.
July 6, 1995	Amendment No. 103 - General Correspondence: Response to FDA Request for Information (comments on proposed analysis plan).
July 20, 1995	Amendment No. 104 - General Correspondence: Request for C,M&C pre-NDA Meeting.
July 25, 1995	Amendment No. 105 - Protocol Amendment.
August 4, 1995	Amendment No. 106 - Safety Report: Follow-up Written Report.
August 7, 1995	Amendment No. 107 - General correspondence: Reply to Comments on Draft Protocol.
August 10, 1995	Amendment No. 108 - General correspondence: Package for CANDA/Protocol 0038 Statistical Plan Mtg.
August 11, 1995	Amendment No. 109 - New Protocol.
August 16, 1995	Correspondence: Desk copy of Protocols for Medical Officer.
August 28, 1995	Amendment No. 110 - Information Amendment.
August 28, 1995	Amendment No. 111 - Protocol Amendment.

DATE	EVENT
August 29, 1995	CANDA meeting.
September 5, 1995	Amendment No. 112 - Addendum to Request for pre-NDA Meeting; Revised Agenda.
September 13, 1995	Amendment No. 113 - Overheads for pre-NDA Meeting.
September 15, 1995	Amendment No. 114 - General correspondence: Response to FDA Request for Information - Clinical.
September 18, 1995	Amendment No. 115 - IND Safety Report: 10-day Written Report.
September 29, 1995	Amendment No. 116 - General Correspondence: CM&C Information - Teleconference report.
October 2, 1995	Amendment No. 117 - General Correspondence: Minutes of August 29, 1995 CANDA meeting.
October 4, 1995	Amendment No. 118 - Protocol Amendment.
October 4, 1995	Pre-NDA Meeting (all items except CM&C). The proposal to submit the FDA under the accelerated approval regulations (21 CFR § 314.510, Subpart H) was accepted. Design of the confirmatory phase IV trial was tentatively agreed upon; design to be finalized and submitted by early 1996.
October 11, 1995	Amendment No. 119 - General correspondence: Minutes of 9/6/95 CMC Pre-NDA Meeting.
October 23, 1995	Amendment No. 120 - Protocol Amendment.
October 30, 1995	Amendment No. 121 - Annual Report.
November 6, 1995	Amendment No. 122 - General Correspondence - Minutes of October 4, 1995, Pre-NDA Meeting.
November 8, 1995	Amendment No. 123 - Safety Report.
November 10, 1995	Amendment No. 124 - New Investigators.

DATE	EVENT
November 21, 1995	Amendment No. 125 - Protocol Amendment.
November 29, 1995	Amendment No. 126 - Safety Report.
December 1, 1995	Amendment No. 127 - Safety Report.
December 8, 1995	Amendment No. 128 - Safety Report.
December 13, 1995	Amendment No. 129 - Change in Protocol.
December 14, 1995	Amendment No. 130 - General Correspondence.
December 12, 1995	Amendment No. 131 - New Protocol.
December 28, 1995	NDA filed with and logged in to FDA.
December 28, 1995	Letter sent to Division of Information Systems Design (HFD-70) with listing of CANDA equipment.
January 3, 1996	Letter sent to Division of Information Systems Design (HFD-70) updating listing of CANDA equipment with information regarding the laptop computers for the medical reviewers.
January 11, 1996	Leslie Vaccari (CSO) called and requested immediate installation of the CANDA hardware (scheduled installation of 1/8/96 cancelled by FDA closure from 1/8 through 1/10 due to weather). CANDA equipment delivered same day by A.A. Khan and J.P. Maile. SAS data sets were hand delivered by R.E. Gibson.
January 11, 1996	Leslie Vaccari informed MAB that page 5/1/32 was missing from the reviewer copy. Replacement page was faxed.
January 16, 1996	Fax from Leslie Vaccari thanking P&U for 1/11/96 CANDA installation.
January 16, 1996	Fax from H.J. DeKoning Gans to FDA. Requested feedback on proposed cut off date (12/31/95) and content of NDA Safety Update.

DATE	EVENT
January 17, 1996	Telephone request from Leslie Vaccari to D.C. Beuving. Requested: immediate CANDA training for medical reviewers; location of specific data within NDA; investigator information on behalf of FDA Division of Scientific Investigations.
January 18, 1996	FDA fax responding to P&U fax of 1/16/96. FDA requests that the safety information provided be consistent with the ISS content submitted in NDA 20-571.
January 18, 1996	A.M. Holt and A.A. Khan provide CANDA training to FDA medical reviewers, Drs. Murgo and Chico. A.M. Holt delivered investigator information requested during 1/17/96 telephone conversation between D.C. Beuving and Leslie Vaccari. Medical reviewers forwarded request for information (summarized in contact report from A.M. Holt dated 1/22/96).
January 19, 1996	Fax from D.C. Beuving to Leslie Vaccari. Provided response to location of patient data requested by Dr. Murgo during 1/17/96 telephone conversation between D.C. Beuving and Leslie Vacari.
January 22, 1996	FDA acknowledgement letter for the NDA (dated 1/22/96). Confirms date of FDA receipt of the application as 12/28/95. Indicates that unless notified to the contrary, the application will be filed in 60 days (calculated as 2/27/96).
January 22, 1996	Dr. Turner (Scientific Investigations) called M.A. Baumgartner to request investigator information and protocol information from pivotal trials.
January 23, 1996	Replacement battery for Dr. Murgo's laptop shipped via overnight mail.
January 25, 1996	Leslie Vaccari called M.A. Baumgartner to request information regarding addresses and DMFs for the Japanese manufacturer and subcontractors for the drug substance. This information is needed to

DATE	EVENT
	support scheduling of the PAI. During the conversation Leslie Vaccari reported that the replacement battery mailed 1/23 is also defective after charging. She requested a second battery.
January 26, 1996	Fax from M.A. Baumgartner to Leslie Vaccari provides requested facility addresses for Yukult Honsha, Sato and Shiratori. Also, addressed DMF issue and provided primary contact at Yukult for DMF 8553.
January 26, 1996	Second replacement battery mailed to Leslie Vaccari for 1/29/96 delivery.
January 29, 1996	Leslie Vaccari called to say that, regarding the EA, it may delete certain format items under the recent guidance to industry (announced in 1/11/96 FR). She suggested that "withdrawal" can be accomplished by superseding via submission of modified EA through the Amendment mechanism.
January 29, 1996	Fax received on EA issue.
January 30, 1996	Submitted response to reviewer questions on the CANDA (from A.M. Holt at the 1/18/96 training session). Submitted as Amendment 001 to the NDA.
January 31, 1996	Submitted response to Dr. Turner's request for protocols, CI information and volume 1.1.
February 1, 1996	Dr. Turner called and identified the CIs to be audited; to request specific CRFs and ADR listings for the CIs identified.
February 6, 1996	Submitted response to Dr. Turner's 2/1/96 request.

DATE	EVENT
February 12, 1996	Telephone contact with Leslie Vaccari. 45 day meeting will be held today. The review team is planning on a June ODAC with an action letter shortly thereafter. Would like revised EA submitted ASAP (prior to 60-day milestone).
February 13, 1996	Telephone contact with Leslie Vaccari. No major deficiencies noted at the 45-day meeting. Planning on a June ODAC. Action letter could come as early as 6/28/96.
February 13, 1996	Dr. Chico contacted A.A. Khan to resolve some CANDA export problems. A telephone conference will be needed to resolve. Message left on Chico's voice mail suggesting call on February 14, 1996.
February 14, 1996	Mailed Amendment 002 to the NDA which contained the revised EA and two corrected pages for Item 3. Corrected CMC pages sent to Detroit office for inclusion in field copy.
February 14, 1996	Contacted Leslie Vaccari to facilitate scheduling a teleconference with Dr. Chico.
February 16, 1996	Fax from Leslie Vaccari to MAB. Comments from Biopharm reviewer. Request for PK data availability in an electronic format.
February 20, 1996	Telephone call with Leslie Vaccari regarding the desire of the biopharm reviewer to get the electronic information detailed in the fax of 2/16. Annette Holt spoke with Dr. Chico regarding the database issues. MAB followed up and offered assistance with respect to producing custom datasets.

DATE	EVENT
February 21, 1996	Larry Schaaf (Clin. PK) spoke with Dr. Williams. It was agreed to send WP 5.2 files immediately for the Item 6 summary and to provide data sets for the "pivotal" phase II studies. Larry Schaaf to call Dr. Williams with estimates of time frame for data sets for Phase II and with results of assessment of feasibility for data builds for the Phase 1 studies conducted by Yakult/ Daiichi/Besselar.
February 22, 1996	MAB spoke with Leslie Vaccari regarding submission of disks. She requested two sets of disks be formally submitted to the application as general correspondence (PK and archival jackets). This and all future letters should be submitted in NDA jackets to expedite processing in the document control room. Letter/disks sent by overnight mail.
February 22, 1996	M.A. Clasby met with FDA inspector Tom Hillary regarding other issues. During the conversation the issue of the CPT-11 PAI was raised. He has tentatively agreed to conduct the PAI starting April 15.
February 23, 1996	A telecon was held between Schaaf, Baumartner and P&U consultants Grasela and Fieldler-Kelly. Reached agreement on additional deliverables.
February 27, 1996	Faxed summary of 2/23/97 teleconference to FDA Project Manager, Leslie Vaccari.
February 28, 1996	Data sets used for structural models plus control streams provided to Dr. Williams by Fiedler-Kelly.
March 1, 1996	Remainder of Phase II data sets sent to Williams by Fieldler-Kelly.
March 7, 1996	Data sets for Phase I studies 0027 and DM111 sent to Williams.

DATE	EVENT
March 7, 1996	Telephone conversation between Dr. Williams and Larry Schaaf. Updated Dr. Williams regarding status of response.
March 8, 1996	Telephone conversation between MAB and Leslie Vaccari. Review continues to proceed smoothly. Modified EA was hand delivered to HFD-357 (Nancy Sager's group). The Microbiology review is back; no deficiencies noted. Trademark review will occur at the end of this month. Leslie OK'd upgrade of the export software for the CANDA. A. Khan will install 3/11/96.
March 8, 1996	From M.A. Clasby from interaction with inspector Tim Hillary: the CPT-11 pre-approval inspection in Kalamazoo is now scheduled to start on Wednesday, April 17th rather than Monday, April 15th. The change has been made so that the PAI for Remifentanil can occur on the 15th and 16th of April.
March 22, 1996	General Correspondence - NDA Safety Update.
April 3, 1996	Amendment 004 - Response to Request for Information - Additional use report forms.
April 4, 1996	Desk copies of updated SAS Datasets (safety and efficacy) sent to Leslie Vaccari. It was agreed with Leslie that Archive copy would follow.
April 5, 1996	PAI scheduled for Kalamazoo changed to begin 4/29/96-4/30/95 per contact between Martha Clasby and Tim Hillary.
April 15, 1996	Amendment 005 - Updated Summary of Efficacy.
April 18, 1996	PAI finished at Sato with 483 issued with 2 minor observations. Approval will be recommended. Shiratori will be inspected the 19th with Yakult finished up by the 26th.

DATE	EVENT
April 18, 1996	The PAI scheduled for 29th and 30th in Kalamazoo will need to be rescheduled at FDA's request (schedule conflict with FDA Inspector, Tim Hillary) for May 7.
May 8, 1996	General Correspondence - Response to Information Request.
May 16, 1996	Amendment 006 - Response to FDA Request for Information - Environmental Assessment.
May 16, 1996	Oncologic Drugs Advisory Committee Brochure - for June 13, 1996 meeting.
May 16, 1996	Sent to desk copies of Oncologic Division Advisory Committee Brochure and copy of revised Environmental Assessment to Ms. Leslie Vaccari as requested during 5/16/96 telephone conversation.
May 16, 1996	Correction to Oncologic Drugs Advisory Committee Brochure. Correction of Protocol.
May 21, 1996	Response to telephone facsimile transmission of May 17, 1996 requesting additional information regarding stability data.
May 21, 1996	General correspondence - response to request for information.
May 28, 1996	Amendment 007 - Correction to Clinical/Statistical Information (Amendment 003). Page 57 of Volume 1, section 8, "Discontinuations Due to Death and/or On-Study Deaths".
May 29, 1996	Amendment 008 - Chemistry, Manufacturing, and Control section - labeling. Copies of proofs for vials, cartons, and blister packs for the product.
May 29, 1996	Desk Copies of Amendment 008 providing proofs of labels for vials, cartons and blister packs. Sample of blister pack with an empty, uncapped vial enclosed.

DATE	EVENT
May 30, 1996	Amendment 009 - Phase 4 Study - Prot. M/6475/0038 mentioned in letter.
May 31, 1996	Correction to Oncologic Drugs Advisory Committee Brochure. Previous package erroneously included the outdated statistical section.
June 4, 1996	Amendment 010 - Response to FDA Request for Information - Environmental Assessment - Revised EA information: Format items 9 and 10.
June 5, 1996	Received missing pages from the background material submitted by the Oncology Division. Also included was a draft of the questions prepared by the division for TUC.
June 6, 1996	General Correspondence - Response to FDA Request for Information - Postmarketing Study Commitments. Prot. M/6475/0038, M/6475/0037, M/6475/0033, M/6475/0062 mentioned in response letter.
June 6, 1996	General correspondence - Response to FDA Request for Information (May 31, 1996 facsimile request) - Microbiologist's Request for Postmarketing Commitments.
June 7, 1996	Amendment 011 - Response to FDA Request for Information. (Per fax of 6/4/96).
June 7, 1996	Oncologic Drugs Advisory Committee (ODAC) - Providing a list of consultants who will be at the 6/13/96 ODAC Meeting as requested by FDA.
June 10, 1996	Amendment 012 - Response to FDA Request for information related to chemistry review.
June 10, 1996	General Correspondence - Request for Teleconference. Letter to Dr. Delap re questions which will be presented at the ODAC.

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DATE	EVENT
June 11, 1996	Amendment 013 - Response to FDA Requested Changes to the Product Labeling. In response to FDA's 5/31, 6/3, 6/7 and 6/10 facsimiles.
June 11, 1996	Proposed news release pending a favorable recommendation at the Oncologic Drugs Advisory Committee Meeting. Also attached is the current draft package insert.
June 12, 1996	Letter to Tracy Acker requesting comments of proposed news release with background information pending favorable recommendation for approval by the ODAC.
June 12, 1996	MACMIS ID #4364 - Letter to K.J. Day providing comments regarding proposed press release in the event of a favorable recommendation for approval by the ODAC.
June 13, 1996	MACMIS ID #4364 - Response to proposed press release following the ODAC meeting on 6/13/96.
June 14, 1996	FDA letter giving approval of NDA 20-571 providing for the treatment of patients with metastatic carcinoma of the colon or rectum whose disease has progressed following 5-FU-based therapy.
June 14, 1996	General Correspondence - Response to FDA Request.

5

6

Exhibit 5

PATENT
55-301M

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re : U.S. Patent 4,604,463

Issued : August 5, 1986

To : Tadashi MIYASAKA, Seigo SAWADA, Kenichiro NOKATA, Eiichi SUGINO, and Masahiko MUTAI

Assignee : KABUSHIKI KAISHA YAKULT HONSHA

For : CAMPTOTHECIN DERIVATIVES AND PROCESS FOR PREPARING SAME

CERTIFICATION

I, LEONARD R. SVENSSON, do hereby certify that this accompanying application for extension of the term of U.S. Patent 4,604,463 under 35 U.S.C. § 156 including its attachments and supporting papers is being submitted as one original and a duplicate copy thereof.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____
Leonard R. Svensson
Reg. No. 30,330

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

Dated: August 12, 1996

LRS/pw

Exhibit 6

PATENT
55-301M

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re : U.S. Patent 4,604,463

Issued : August 5, 1986

To : Tadashi MIYASAKA, Seigo SAWADA, Kenichiro NOKATA, Eiichi SUGINO, and Masahiko MUTAI

Assignee : KABUSHIKI KAISHA YAKULT HONSHA

For : CAMPTOTHECIN DERIVATIVES AND PROCESS FOR PREPARING SAME

DECLARATION ACCOMPANYING APPLICATION UNDER 35 U.S.C. § 156 FOR EXTENSION OF PATENT TERM

I, LEONARD R. SVENSSON, do hereby declare:

I am a patent attorney authorized to practice before the United States Patent and Trademark Office and I have been appointed as attorney by the Patent Assignee, KABUSHIKI KAISHA YAKULT HONSHA, with regard to this application for extension of the term of U.S. Patent 4,604,463 and to transact all business in the U.S. Patent and Trademark Office in connection therewith.

I have reviewed and understand the contents of the accompanying application being submitted pursuant to 37 C.F.R. § 1.740.

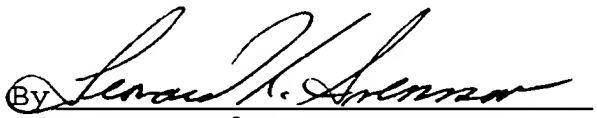
I believe the patent is subject to extension pursuant to 37 C.F.R. § 1.710.

I believe that an extension of the length claimed is justified under 35 U.S.C. § 156 and applicable regulations.

I believe the patent for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 37 C.F.R. § 1.720.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 

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Dated: August 12, 1996